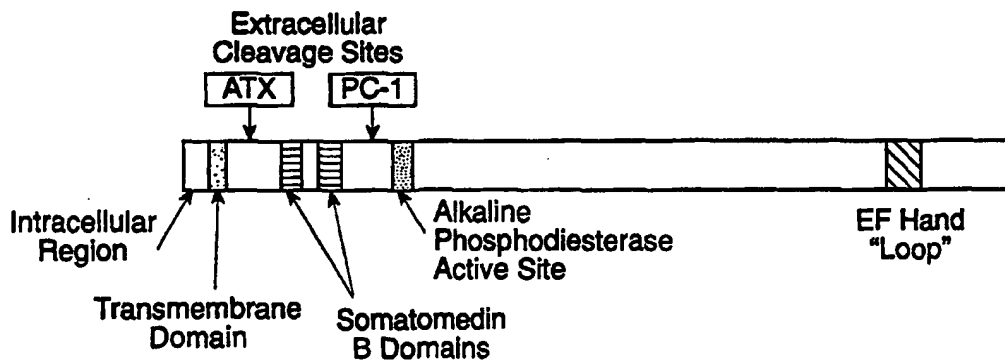


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(54) Title: AUTOTAXIN: MOTILITY STIMULATING PROTEIN USEFUL IN CANCER DIAGNOSIS AND THERAPY**(57) Abstract**

The present invention relates, in general, to autotaxin. In particular, the present invention relates to a DNA segment encoding autotaxin; recombinant DNA molecules containing the DNA segment; cells containing the recombinant DNA molecule; a method of producing autotaxin; antibodies to autotaxin; and identification of functional domains in autotaxin.

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AUTOTAXIN: MOTILITY STIMULATING PROTEIN USEFUL
IN CANCER DIAGNOSIS AND THERAPY

This application is a continuation-in-part of
application serial no. 08/249,182 filed May 25, 1994,
which is a continuation-in-part of application serial no.
07/822,043 filed on Jan. 17, 1992.

Field of the Invention

The present invention relates, in general, to a
motility stimulating and compositions comprising the same.
In particular, the present invention relates to a purified
form of the protein and peptides thereof, for example,
autotaxin (herein alternative referred to as "ATX"); a DNA
segment encoding autotaxin; recombinant DNA molecules
containing the DNA segment; cells containing the
recombinant DNA molecule; a method of producing autotaxin;
antibodies to autotaxin; and methods of cancer diagnosis
and therapy using the above referenced protein or peptides
thereof and DNA segments.

Background of the Invention

Cell motility plays an important role in
embryonic events, adult tissue remodeling, wound healing,
angiogenesis, immune defense, and metastasis of tumor
cells (Singer, 1986). In normal physiologic processes,
motility is tightly regulated. On the other hand, tumor
cell motility may be aberrantly regulated or
autoregulated. Tumor cells can respond in a motile
fashion to a variety of agents. These include host-
derived factors such as scatter factor (Rosen, et al.,
1989) and growth factors (Kahan, et al., 1987; Stracke, et
al.; Tamm, et al., 1989; Wang, et al. 1990; and Jouanneau,
et al. 1991), components of the extracellular matrix
(McCarthy, et al. 1984), and tumor-secreted or autocrine
factors (Liotta, et al. 1988; Ruff, et al. 1985; Atnip, et
al. 1987; Ohnishi, et al. 1990; Silletti, et al. 1991; and
Watanabe, et al. 1991).

Many types of host-derived soluble factors act

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° in a paracrine fashion to stimulate cell locomotion. Motility-stimulating proteins called "scatter factors" have been identified which are produced by embryonic fibroblasts and by smooth muscle cells (Stoker, et al. 1987). Scatter factors stimulate random and directed
5 motility by epithelial cells, keratinocytes, vascular endothelial cells and carcinoma cells (Stoker, et al. 1987; Rosen, et al. 1990; and Weidner, et al. 1990), but not fibroblasts. In addition, a number of host-secreted growth factors have been demonstrated to stimulate
10 motility in tumor cells, including nerve growth factor (Kahan, et al. 1987) insulin-like growth factor-I (Stracke, et al. 1988), interleukin-6 (Tamm, et al. 1989), interleukin-8 (Wang, et al. 1990), and acidic fibroblast growth factor (Jouanneau, et al. 1991). These paracrine
15 factors may influence "homing" or the directionality of tumor cell motility.

In contrast to these host-derived factors, many types of tumor cells have been found to produce proteins termed "autocrine motility factors" which stimulate
20 motility by the same tumor cells which make the factor (Liotta, et al. 1986). Autocrine motility factors are not specific for a given type of cancer cell but have a wide spectrum of activity on many types of cancer cells (Kohn, et al. 1990), with little effect on normal fibroblasts or
25 leukocytes.

Autocrine motility factors identified to date act through cell-surface receptors (Stracke, et al. 1987; Nabi, et al. 1990; Watanabe, et al. 1991) resulting in pseudopodial protrusion (Guirguis, et al. 1987) leading to
30 both random and directed migration (Liotta, et al. 1986; Atnip, et al. 1987; Ohnishi, et al. 1990).

Prior studies of human A2058 melanoma cells have demonstrated that these cells are a particularly rich source of autocrine motility factors. An autocrine
35 motility factor with a molecular mass of approximately 60

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° kDa has been previously isolated from the conditioned media of these cells. (Liotta, et al. 1986). Similar tumor cells derived or induced factors with the same molecular weight have subsequently been reported and purified by several investigators (Atnip, et al. 1987; 5 Schnor, et al. 1988; Ohnishi, et al. 1990; Silletti, et al. 1991; Watanabe et al. 1990). Such factors are thought to play a key role in tumor cell invasion.

Most of the motility factors identified to date have not been purified to homogeneity and have not been sequenced. The novel tumor motility factor of the present invention, named herein as autotaxin ("ATX"), has been purified and verified to be a homogeneous sample by two-dimensional gel electrophoresis. The protein of the present invention is unique from any previously identified 15 or purified motility factor. The molecular size of ATX is about 125 kilo Daltons ("kDa") and it has an isoelectric point of approximately 7.7. ATX stimulates both random and directed migration of human A2058 melanoma cells at picomolar concentrations. The activity of the ATX factor 20 is completely sensitive to inhibition by pertussis toxin. No significant homology has been found to exist between the protein of the invention and any mammalian protein including previous factors known to stimulate cell motility.

25 There is a great clinical need to predict the aggressiveness of a patient's individual tumor, to predict the local recurrence of treated tumors and to identify patients at high risk for development of invasive tumors. The present invention provides a functional marker which 30 is functionally related to the invasive potential of human cancer. The invention further provides an assay for this secreted marker in body fluids, or in tissues. The assay of the invention can be used in the detection, diagnosis, and treatment of human malignancies and other 35 inflammatory, fibrotic, infectious or healing disorders.

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SUMMARY OF THE INVENTION

The present invention relates, generally, to a motility stimulating protein and corresponding peptides thereof, and to a DNA segment encoding same. A human cDNA clone encoding a tumor cell motility-stimulating protein, herein referred to as autotaxin or "ATX", reveals that this protein is an ecto/exoenzyme with significant homology to the plasma cell membrane differentiation antigen PC-1. ATX is a 125 kDa glycoprotein, previously isolated from a human melanoma cell line (A2058), which elicits chemotactic and chemokinetic responses at picomolar to nanomolar concentrations.

It is a specific object of the present invention to provide autotaxin and peptide fragments thereof.

It is a further object of the present invention to provide a DNA segment that encodes autotaxin and a recombinant DNA molecule comprising same. It is a further object of the present invention to provide a cell that contains such a recombinant molecule and a method of producing autotaxin using that cell.

Another object of the present invention is the identification of a transmembrane domain of the human liver autotaxin protein and its apparent absence in tumorous forms of autotaxin. The tumorous form of autotaxin appears to be a secreted protein. The present invention relates to utilization of the different sites of localization for diagnosis and prognosis of the stages of tumor progression. Further, the invention relates to treatment methods, designed to advantageously block the secreted form of autotaxin activity while having little effect on the membrane-bound form of autotaxin.

Yet another object of the present invention relates to the identification of a highly variable region within the autotaxin gene, called a "hot spot". The variations in sequence apparently result in mutations, insertions, deletions and premature termination of

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translation. The present invention relates to manipulating this region so as to alter the activity of the protein. Further, the hot spot can serve as a marker in tumor diagnosis differentiating between different forms of the autotaxin protein.

5 It is yet another object of the present invention to provide a method of purifying autotaxin.

It is a further object of the present invention to provide cloned DNA segments encoding autotaxin and fragments thereof. The cDNA encoding the entire autotaxin protein contains 3251 base pairs, and has an mRNA size of approximately 3.3 kb. The full-length deduced amino acid sequence of autotaxin comprises a protein of 915 amino acids. Database analysis of the ATX sequence revealed a 45% amino acid identity (including 30 out of 33 cysteines) with PC-1, a pyrophosphatase/type I phosphodiesterase expressed on the surface of activated B cells and plasma cells. ATX, like PC-1, was found to hydrolyze the type I phosphodiesterase substrate p-nitrophenyl thymidine-5'-monophosphate. Autotaxin now defines a novel motility-regulating function for this class of ecto/exo-enzymes.

Further objects and advantages of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

25 Figure 1. Fractionation of ATX by hydrophobic interaction. A 200 ml sample of A2058 conditioned media was chromatographed on a 200 mL column of phenyl Sepharose-4B. Buffer A was 50 mM Tris (pH 7.5), 5% methanol, and 1.2 M ammonium sulfate. Buffer B was 50 mM Tris (pH 7.5), 5% methanol and 50% ethylene glycol. The gradient (----) represents a double linear gradient with decreasing ammonium sulfate (1.2 to 0.0 M) and increasing ethylene glycol (0 to 50%). Absorbance was monitored at 280 nm (—) and indicated that most of the protein did not bind to the column. Ten ml fractions were assayed for

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- motility stimulating capacity using the Boyden Chamber assay (o). The peak of motility activity occurred between 900 and 1050 minutes, ~ 12% of the gradient.

Figure 2. Isolation of ATX by lectin affinity chromatography. 20 ml portions of the phenyl Sepharose activity peak were affinity purified on a 40 ml Concanavalin A Affi-Gel column. The bound components were eluted with a step gradient (----) of methyl α -D-mannopyranoside (0.0 mM, 10 mM, and 500 mM) in a buffer consisting of 0.05 M Tris (pH 7.5), 0.1 M NaCl, 0.01 M CaCl₂, and 20% ethylene glycol. Absorbance was monitored at 280 nm (_____) and indicated that the majority of the protein components did not bind to the column. Motility was assayed in 10 mL fractions (...o...) and was found predominantly in the 500 mM elution concentration. One of seven chromatographic runs is shown.

Figure 3. Purification of ATX by weak anionic exchange chromatography. Approximately 30% of the activity peak eluted from the Con A affinity column was applied to a ZORBAX BioSeries-WAX column. The bound components were eluted with an NaCl gradient (----) in a buffer consisting of 10 mM Tris (pH 7.5) and 30% ethylene glycol. Motility (o) was assayed in 1.0 ml fractions. The peak of activity eluted in a discrete but broad region in the shallow portion of the gradient. Absorbance was monitored at 230 nm (____). The majority of the protein components not associated with activity bound strongly to the column were eluted at 1.0 M NaCl. One of two chromatographic runs is shown.

Figure 4. Purification of ATX by molecular sieve exclusion chromatography. The entire activity peak eluted from the weak anion exchange column was applied to a series of TSK columns (4000SW, 4000SW, 3000SW, and 2000SW, in this order). Proteins were eluted in a buffer consisting of 0.1M NaPO₄ (pH 7.2) with 10% methanol and 10% ethylene glycol. Two major protein peaks were evident

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- ° by monitoring the absorbance at 235 nm (____). Motility (...o...) was assayed in 0.4 ml samples and found predominantly in the first, smaller, protein peak.

Figure 5. Final purification of ATX by strong anionic exchange chromatography. Approximately 15% of the activity peak from the molecular sieve exclusion series was applied to a Pro-Pac PA1 column. Protein which bound to the column was eluted with a NaCl gradient (----) in a buffer consisting of 10 mM Tris (pH 7.5), 5% methanol and 20% ethylene glycol. Absorbance was monitored at 215 nm (____). Motility activity was assayed in 1.0 ml fractions at two different dilutions: 1/5 (...o....) or 1/15 (.__o.__). Activity was found to correspond to a double protein peak in the central region of the gradient.

Figure 6A, 6B and 6C. Protein components associated with the activity peaks from various stages of purification. The activity peak from each chromatographic fractionation was pooled, concentrated and analyzed by SDS-polyacrylamide gel electrophoresis. Molecular weight standards are in Lane 1 for each panel. Panel 6A) 8-16% gradient gel of the first three purification steps, run under non-reducing conditions. Lane 2 is an aliquot of the pooled activity peak eluted from the phenyl sepharose fractionation. Lane 3 is an aliquot of the pooled activity peak eluted from the Con A affinity purification. Lanes 4 and 5 show the "peak" and "shoulder" of activity fractionated by weak anion exchange chromatography (Figure 3). Panel 6B) 7% gel of the activity peak fractionated by molecular sieve exclusion chromatography. Lanes 2 and 3 show the protein separation pattern of the total pooled activity peak when the gel was run under non-reducing and reducing conditions, respectively. Panel 6C) 8-16% gradient gel of the final strong anionic exchange chromatographic separation, run under non-reducing conditions. Lane 2 comprises ~1% of the total pooled activity peak eluted from the column.

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Figure 7. Two-dimensional gel electrophoresis of ATX. Purified ATX (Figure 6, Panel C) was subjected to non-equilibrium isoelectric focusing (5 hr. at 500v), then applied to a 7.5% SDS-polyacrylamide gel for the second dimension. The pH separation which resulted was measured in 0.5 cm samples of concurrently run tube gels and is shown at the top. Molecular weight standards for the second dimension are shown on the right. This analysis reveals a single component with $pI = 7.7 \pm 0.2$ and $M_r = 120,000$.

Figure 8. Dilution curve of ATX. Purified ATX (Figure 6, Panel C) was serially diluted and tested for motility-stimulating activity. The result, with unstimulated background motility subtracted out, shows that activity is half-maximal at ~ 500 pM ATX.

Figure 9. Pertussis toxin (PT) sensitivity of ATX. A2058 cells were pre-treated for 1 hr. prior to the start of the motility assay with $0.5 \mu\text{g/ml}$ PT in 0.1% BSA-DMEM or with 0.1% BSA-DMEM alone (for untreated control). The motility activity stimulated by purified ATX (Figure 6, Panel C) was then assessed for the two treatment groups. The result, expressed as cells/HPF \pm S.E.M. with unstimulated background motility subtracted out, reveals profound inhibition of PT-treated cells (hatched) compared to untreated cells (solid). PT had no effect on cell viability. S.E.M.'s were $< 10\%$.

Figure 10. Checkerboard analysis of ATX-stimulated motility. Varying dilutions of autotaxin were added to the upper chamber with the cells and/or to the lower chamber, as shown. Motility response, expressed as cells/HPF \pm S.E.M., was assessed for each point in the checkerboard.

Figure 11. Purification of ATX peptides on HPLC. ATX, purified to homogeneity by strong anionic exchange chromatography, was sequentially digested by cyanogen bromide, subjected to reduction and

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pyridylethylation, and digested by trypsin. The resulting peptides were purified on an Aquapore RP300 C-8 reverse phase column using a (0-70)% acetonitrile gradient in 0.1% trifluoroacetic acid (----). The absorbance was monitored at 215 nm (____) and peaks were collected. Seven peaks, chosen at random for N-terminal amino acid sequence analysis, are shown with appropriate numbers.

Figure 12. Cloning Strategy, schematically depicted.

Figure 13. Schematic Diagram of autotaxin gene region.

For A2058: 4C11 is the original DNA clone obtained by screening an A2058 cDNA expression library in λ gt11 with anti-peptide ATX-102. Upstream ATX peptide sequences were utilized for PCR amplification of A2058 mRNA, using the technique of reverse transcription/PCR. These peptides include ATX-101, ATX-103, and ATX-224. The approximate localization of each of peptide was obtained by matching the peptide with its homologous region on PC-1.

For N-tera 2D1, a λ gt10 cDNA library was amplified and the cDNA inserts were isolated. PCR amplification, based on homologies with A2058 sequence, was utilized for DNA sequencing.

For normal human liver, a mRNA from liver was amplified with 5'RACE using primers from the known ATX-224 region of A2058 and N-tera 2D1.

Figure 14. Schematic match-up of ATX peptides with putative A2058 protein sequence.

Figure 15. Schematic match-up of ATX peptides with putative N-tera 2D1 protein sequence.

Figure 16: ATX Treatment with PGNase F. Partially purified ATX was treated with 60 mU/ml PNGase F at 37°C for 16 hr under increasingly denaturing conditions. The treated ATX samples were separated by SDS polyacrylamide gel electrophoresis run under reducing

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° conditions and stained with Coomassie blue G-250. Lane 1 contains untreated ATX (arrow) with no enzyme added. Lane 2 contains the reaction mixture run under non-denaturing conditions (50 mM tris/10% ethylene glycol, pH 7). Lanes 3 and 4 have added 0.1 M β -mercaptoethanol and 0.5%
5 Nonidet-P40, respectively. Lanes 5 and 6 contain the reaction mixtures in which the ATX sample was first boiled for 3 min in 0.1% SDS with (lane 6) or without (lane 5) 0.1 M β -mercaptoethanol, then had 0.5% Nonidet-P40 added to prevent enzyme denaturation. The enzyme can be
10 detected as an ~44 kDa band in lanes 2-6.

Figure 17: Effect of varying concentrations of PNGase F on ATX molecular weight and motility-stimulating activity. Partially purified ATX was treated with various concentrations (range 0 - 60 mU/ml, shown on horizontal
15 axis) of PNGase F at 37°C for 16 hr. Figure 17A shows the effect of the different treatments on ATX molecular weight. At concentrations of enzyme ≥ 30 mU/ml, the deglycosylation reaction appears to be complete. Figure 17B shows the effect of the identical reaction mixtures on
20 motility-stimulating capacity (immediately below the corresponding protein band of Figure 17A). There is no significant difference between any of the treatment groups.

Figure 18: Comparison of amino acid sequences
25 of ATX and PC-1. The amino acid sequences of ATX and PC-1 are compared. Amino acid identity is indicated by a vertical line between the sequences. The location of the putative transmembrane/signal sequence is shown by a solid line. The two somatomedin B domains are identified by
30 dashed lines. The putative phosphodiesterase active site is indicated by emboldened lines. The loop region of a single EF hand loop region is identified with double lines. The presumed cleavage site for each protein is indicated with arrows.

35 Figure 19: Domain structure of ATX and PC-1.

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- Putative domains are indicated for the two homologous proteins, ATX and PC-1.

DETAILED DESCRIPTION OF THE INVENTION

Tumor cell motility is a critical component of invasion and metatasis, but the regulation of this motility is still poorly understood. At least some tumor cells secrete autocrine motility factors (AMF's) that stimulate motility in the producing cells. Like the analogous autocrine growth factors, these AMF's allow tumor cells independence from the host in this important component of the metastatic cascade. One AMF, autotaxin (ATX), has recently been purified to homogeneity from the human melanoma cell line, A2058 (Stracke, et al., 1992). The purified protein was enzymatically digested and the peptide fragments were separated by reverse phase HPLC. A number of these peptides have been sequenced by standard Edman degradation (Table 6) from different purifications and different enzymatic digestion. Sequence information, obtained initially on 19 purified tryptic peptides, confirmed that the protein is unique with no significant homology to growth factors or previously described motility factors. These peptide sequences have now been used as the basis for identifying and sequencing the cDNA clone for ATX. The present invention comprises an amino acid sequence of ATX as well as a nucleic acid sequence coding for the ATX protein.

TABLE 6. PEPTIDE SEQUENCES FOR AUTOTAXIN.

	PEPTIDE NO.	AMINO ACID SEQUENCE	SEQ ID: NO:
30	ATX-18	WHVAR	SEQ ID NO:1
	ATX-19	PLDVYK	SEQ ID NO:2
	ATX-20	YPAFK	SEQ ID NO:3
	ATX-29	PEEVTRPNYL	SEQ ID NO:5
35	ATX-34B	RVWNYFQR	SEQ ID NO:38

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°	ATX-41	HLLYGRPAVLY	SEQ ID NO:29
	ATX-48	VPPFENIELY	SEQ ID NO:7
	ATX-59	TFPNLYTFATGLY	SEQ ID NO:32
	ATX-100	GGQPLWITATK	SEQ ID NO:8
5	ATX-101/223A	VNSMQTVFVGYGPTFK	SEQ ID NO:9
	ATX-102	DIEHLTSLDFFR	SEQ ID NO:10
	ATX-103	TEFLSNYL TNVDDITLVPETLGR	SEQ ID NO:11
	ATX-104	VNVISGPIDDYDYDGLHDTEDK	SEQ ID NO:33
	ATX-204	MHTARVRD	SEQ ID NO:39
10	ATX-205	FSNNAKYD	SEQ ID NO:40
	ATX-209	VMPNIEK	SEQ ID NO:41
	ATX-210	TARGWECT	SEQ ID NO:42
	ATX-212	(N)DSPWT(N)ISGS	SEQ ID NO:43
	ATX-214	LRSCGTHSPYM	SEQ ID NO:44
15	ATX-215/34A	TYLHTYES	SEQ ID NO:45
	ATX-213/217A	AIANLTCKKPDQ	SEQ ID NO:46
	ATX-216	IVGQLMDG	SEQ ID NO:47
	ATX-218/44	TSRSYPEIL	SEQ ID NO:48
	ATX-223B/24	QAEVSSVPD	SEQ ID NO:49
20	ATX-224	RCFELQEAGPPDDC	SEQ ID NO:50
	ATX-229	SYTSCCHDFDEL	SEQ ID NO:51
	ATX-244/53	QMSYGFLFPPYLSSSP	SEQ ID NO:52

ATX is a glycosylated protein due to its high
 25 affinity for concanavalin A and amino acid sequence
 analysis of the ATX peptides. ATX has been demonstrated
 to be a 125kDa glycoprotein whose molecular weight reduced
 to 100-105kDa after deglycosylation with N-glycosidase F.
 The calculated molecular weight of the cloned protein is
 30 100kDa (secreted form) or 105kDa (full length protein).
 Based on amino acid composition, the estimated pI is 9.0
 which is higher than the pI determined by 2-D gel
 electrophoresis analysis (7.7-8.0) of purified ATX. This
 difference can be explained by the presence of sialic acid
 35 residues on the sugar moieties.

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Autotaxin is secreted by A2058 human melanoma cells cultured in low abundance in serum-free conditioned medium. Autotaxin is a potent new cytokine with molecular mass 125 kDa which has been purified to homogeneity from the conditioned medium of the human melanoma cell line, A2058, utilizing sequential chromatographic methods as described herein. This new cytokine, termed autotaxin (ATX), is a basic glycoprotein with pI ~ 7.8. ATX is active in the high picomolar to low nanomolar range, stimulating both chemotactic and chemokinetic responses in the ATX-producing A2058 cells as well as other tumor cells. This motile response is abolished by pretreatment of the cells with pertussis toxin. ATX may therefore act through a G protein-linked cell surface receptor. These characteristics distinguish ATX from several small growth factors and interleukins which are implicated in tumor cell motility (Stracke et al., 1988; Ruff et al., 1985; Maciag et al., 1984; Gospodarowicz, 1984; Van Snick, 1990; Yoshimura 1987).

The protein of the present invention, which in one embodiment is derived from A2058 human melanoma cells, can be prepared substantially free from proteins with which it is normally associated using, for example, the purification protocol disclosed herein. Alternatively, the protein of the present invention can be prepared substantially free from proteins, by cloning and expressing the cDNA encoding autotaxin as disclosed herein.

A large volume of serum-free conditioned medium from appropriate producer cells (e.g., tumor cells) is collected and concentrated approximately 500-fold. This concentrated conditioned medium is then separated from other contaminating proteins by techniques that rely on the chemical and physical characteristics of the protein. These include the molecular weight, relative hydrophobicity, net charge, isoelectric focusing point,

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° and the presence of lectin-binding sugar residues on the protein.

Alternatively, the protein, or functional portion thereof, can be synthesized using chemical or recombinant means.

5 The protein of the present invention has a potent biological activity. Purified ATX is active in the picomolar range and 1 unit of activity corresponds to a concentration of approximately 500 pM as assessed by the cell motility assay described herein and elsewhere
10 (Stracke et al., 1989).

The protein of the present invention has a molecular size, as determined by two dimensional gel electrophoresis, of from 120 to 130 kDa, or more specifically, about 125 kDa. Further, the protein of the
15 present invention can have a pI in the range of 7.5 to 8.0, preferably, approximately 7.7. The present invention relates to autotaxin and peptides thereof having cell motility properties as described herein. These proteins and peptides thereof can be produced by isolation from a
20 natural host or isolation as an expression product from a recombinant host.

The present invention also relates to a DNA segment coding for a polypeptide comprising an amino acid sequence corresponding to ATX, or a unique portion of such a
25 sequence (unique portion being defined herein as at least 5, 10, 25, or 50 amino acids). In one embodiment, the DNA segment encodes any one of the amino acid sequences shown in SEQ ID NO:1 to SEQ ID NO:11 and SEQ ID NO:26 to SEQ ID NO:33. Another embodiment uses larger DNA fragments
30 encoding amino acid sequences shown in SEQ ID NO:34, SEQ ID NO: 36 and SEQ ID NO:38. The entire coding region for autotaxin can also be used in the present invention shown in SEQ ID NO:66 through SEQ ID NO:69.

In another embodiment, the present invention relates
35 to a recombinant DNA molecule comprising a vector (for

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° example plasmid or viral vector) and a DNA segment coding for a polypeptide corresponding to ATX, as can be prepared by one skilled in the art. Preferably, the coding segment is present in the vector operably linked to a promoter. The present invention also relates to a recombinant
5 protein produced from a host cell expressing a cDNA containing a coding region of ATX. Examples of ATX cDNAs from a variety of sources have been cloned and can be used for expression, including *inter alia* A2058 carcinoma cells, N-tera 2D1 cells and human liver.

10 In a further embodiment, the present invention relates to a cell containing the above-described recombinant DNA molecule. Suitable host cells include procaryotic cells (such as bacteria, including E. coli) and both lower eucaryotic cells (for example, yeast) and
15 higher eucaryotic cells (for example, mammalian cells). Introduction of the recombinant molecule into the host cells can be effected using methods known in the art.

In another embodiment, the present invention relates to a method of producing a polypeptide having an amino
20 acid sequence corresponding to ATX. The method comprises culturing the above-described cells under conditions such that the DNA segment is expressed, and isolating ATX thereby produced.

In a further embodiment, the present invention
25 relates to an antibody having affinity for ATX or peptide fragments thereof. The invention also relates to binding fragments of such antibodies. In one preferred embodiment, the antibodies are specific for ATX peptides having an amino acid sequence set forth in one of SEQ ID
30 NO:1 through SEQ ID NO:11 and SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO: 36 and SEQ ID NO:38 through SEQ ID NO:52. In addition, the antibodies may recognize an entire autotaxin protein.

Antibodies can be raised to autotaxin or its fragment
35 peptides, either naturally-occurring or recombinantly

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produced, using methods known in the art.

The ATX protein and peptide fragments thereof described above can be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as carrier proteins. In particular, ATX fragments can be fused or covalently linked to a variety of carrier proteins, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See for example, Harper and Row, (1969); Landsteiner, (1962); and Williams et al., (1967), for descriptions of methods of preparing polyclonal antisera. A typical method involves hyperimmunization of an animal with an antigen. The blood of the animal is then collected shortly after the repeated immunizations and the gamma globulin is isolated.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts. Description of techniques for preparing such monoclonal antibodies may be found in Stites et al., and references cited therein, and in particular in Kohler and Milstein (1975), which discusses one method of generating monoclonal antibodies.

In another embodiment, the present invention relates to an oligonucleotide probe synthesized according to the sense or antisense degenerative sequence set forth in one of SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:33, SEQ ID NO:39 through SEQ ID NO:52, and SEQ ID NO:55 through SEQ ID NO:65.

Protein database searches of this sequence revealed a 45% amino acid identity with the plasma cell membrane marker protein, PC-1. ATX and PC-1 appear to share a number of domains, including two somatomedin B domains, the loop region of an EF hand, and the enzymatic site of type I phosphodiesterase/ nucleotide pyrophosphatase. Like PC-1, ATX hydrolyzes p-nitrophenyl thymidine-5' - monophosphate, a type 1 phosphodiesterase substrate. This

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- ° enzymatic function of ATX suggests a newly identified function for ecto/exo-enzymes in cellular motility.

In a further embodiment, the present invention relates to a method of diagnosing cancer metastasis and to a kit suitable for use in such a method. Preferably,
5 antibodies to ATX can be used in, but not limited to, ELISA, RIA or immunoblots configurations to detect the presence of ATX in body fluids of patients (e.g. serum, urine, pleural effusions, etc.). These antibodies can also be used in immunostains of patient samples to detect the
10 presence of ATX.

In yet another embodiment, the present invention relates to *in vivo* and *in vitro* diagnostics. ATX may be radiolabelled, by means known to one skilled in the art, and injected in cancer patients with appropriate ancillary
15 substances also known to one skilled in the art, in order to ultimately detect distant metastatic sites by appropriate imagery. The level of ATX in tissue or body fluids can be used to predict disease outcomes and/or choice of therapy which may also include ATX inhibitors.

20 In a further embodiment, the present invention relates to a treatment of cancer. ATX antibodies can be cross-linked to toxins (e.g., Ricin A), by means known to one skilled in the art, wherein the cross-linked complex is administered to cancer patients with appropriate
25 ancillary agents by means known to one skilled in the art, so that when the antibody complex binds to the cancer cell, the cell is killed by the cross-linked toxin.

In another embodiment, the different localizations of the normal versus tumorous forms of the ATX proteins
30 within the tissue can be used as a tool for diagnosis and prognosis. The stage of disease progression can be monitored by elevated levels of ATX in the extracellular space as opposed to its normal cell membranes association. In addition, treatment methods for control of tumor
35 progression can be designed to specifically block the

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° activity of the secreted form of ATX. Such methods would have a preferential effect upon secreted ATX during tumor progression while not effecting normal ATX formation.

Yet another embodiment utilizes the hot spot located in the region from approximately nucleotides 1670 through
5 1815, as a marker gene for identification of tissues carrying a tumorous form of ATX.

The present invention is described in further detail in the following non-limiting examples.

EXAMPLES

10 The following protocols and experimental details are referenced in the Examples that follow:

Materials. The polycarbonate Nuclepore membranes and the 48-well microchemotaxis chambers were obtained from Neuro Probe, Inc. Pertussis toxin (PT), ethylene glycol
15 (biotechnology grade), methyl α -D-mannopyranoside were obtained from commercial vendors. The ampholyte, pH 3-10 Bio-Lyte and pH 8-10 Bio-Lyte, were obtained from Bio-Rad. Phenyl Sepharose CL-4B; affi-Gel concanavalin A; ZORBAX BioSeries-WAX (weak anion exchange) column (9.4mm x 24cm);
20 Spherogel-TSK 4000SW, 3000SW and 2000SW columns (each 7.5mm x 30cm); the Pro-Pac PA1 (4 x 50mm) strong anion exchange column; the Aquapore RP300 C-8 reverse phase column (220 x 2.1mm); and the AminoQuant C-18 reverse phase column (200 x 2.1mm) were also obtained from
25 commercial sources.

Affi-Gel 10 affinity resin was from Bio-Rad. The GeneAmp PCR Reagent kit with AmpliTaq and the GeneAmp RNA PCR kit were purchased from Perkin-Elmer. The 5' RACE kit came from Gibco BRL Life Technologies, Inc. The
30 p-nitrophenyl thymidine-5'-monophosphate was obtained from Calbiochem Biochemicals.

Ethylene glycol (biotechnology grade) was from Fisher Biochemicals (Pittsburg, PA). Peptide N-glycosidase F ("PNGase F"), O-glycosidase, neuraminidase (*Arthrobacter ureafaciens*), and swainsonine ("Swn") came from
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Boehringer-Mannheim (Indianapolis, IN). 1-Deoxymannojirimycin ("dMAN"), and N-methyl-1-deoxynojirimycin ("NMdNM") were from Oxford GlycoSystems, Inc. (Rosedale, NY). Biotinylated concanavalin A, HRP-conjugated streptavidin, and HRP-conjugated goat anti-rabbit immunoglobulin were purchased from Pierce Chemicals (Rockford, IL). Polyvinyl pyrrolidone-free polycarbonate membranes and the microchemotaxis chamber were from NeuroProbe, Inc. (Cabin John, MD).

Cell Culture. The human melanoma cell line A2058, originally isolated by Todaro (Todaro et al., 1980), was maintained as previously described by Liotta (Liotta et al., 1986). The N-tera 2 (D1 clone) was a kind gift from Dr. Maxine Singer, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health and was maintained as described (Andrews, P.W., Goodfellow, P.N. and Bronson, D.L. (1983) *Cell surface characteristics and other markers of differentiation of human teratocarcinoma cells in culture.*).

Production of Autotaxin. A2058 cells were grown up in T-150 flasks, trypsinized, and seeded into 24,000 cm² cell factories at a cell density of 1×10^{10} cells/factory. After 5-6 days, the serum-containing medium was removed and the cells were washed with DPBS. The factories were maintained in DMEM without phenol red, supplemented with 4 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, 5 µg/ml crystallized bovine serum albumin, 10 µg/ml bovine insulin, and 1 µM aprotinin. Culture supernatants were harvested every 3 days, frozen at -40°C and replaced with fresh serum-free medium. Each cycle of supernatant was tested for ATX production with a cell motility assay detailed below. Typically, a cell factory continued to be productive for 9-11 of these cycles.

After accumulation of approximately 45-60 L of supernatant, the culture supernatants were thawed and concentrated down to 2-2.5 L using an Amicon S10Y30 spiral

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° membrane ultrafiltration cartridge. This supernatant was further concentrated in an Amicon high performance ultrafiltration cell using Diaflo membranes. The final volume achieved from 100-200 L of conditioned medium was typically 250-400 ml. All ultrafiltrations were performed at 4°C.

5 Cell Motility Assays. Purification of autotaxin was monitored by testing the motility-stimulating capacity of the fractions collected from the columns. These fractions were in buffers unsuitable for a chemotaxis assay so each fraction had to be washed into an appropriate buffer, i.e., 0.1% (w/v) BSA in DPBS containing calcium and magnesium. This dialysis was performed by adding aliquots of each fraction to be tested into Centricon-30™ ultrafiltration tubes, which retain molecular species larger than 30,000 daltons.

15 The assay to determine motility was performed in triplicate using a 48-well microchemotaxis chamber as described elsewhere in detail (Stracke et al., 1987; Stracke, et al., 1989). The Nuclepore™ membranes used in these modified Boyden chambers were fixed and stained with Diff-Quik.™ Chemotaxis was quantitated either by reading the stained membranes with a 2202 Ultrosan laser densitometer or by counting 5 randomly chosen high power fields (HPF) under light microscopy (400 x) for each replicate. Densitometer units (wavelength - 633 nm) have been shown to be linearly related to the number of cells per HPF (Taraboletti, 1987; Stracke, et al., 1989). Typically, unstimulated motility (background) corresponded to 5-10 cells/HPF and highly responding cells to 70-100 cells/HPF above unstimulated background (i.e., 75-110 total cells/HPF).

25 For experiments using PT, the toxin was pre-incubated with the cells for 1-2 hr. at room temperature prior to the assay and maintained with the cells throughout the assay (Stracke, et al., 1987). The treated cells were

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° tested for their motility response to the chemoattractant as well as for unstimulated random motility.

Purification of Autotaxin. Ammonium sulfate, to a final concentration of 1.2 M, was added to the concentrated A2058 conditioned medium for 1 hr. at 4°C. The solution
5 was spun in a RC2-B Ultraspeed Sorvall centrifuge at 10,000 x g for 15 min. Only the supernatant had the capacity to stimulate motility.

In the first step, the sample was fractionated by hydrophobic interaction chromatography using 200 ml phenyl
10 Sepharose CL-4B column equilibrated into 50 mM Tris (pH 7.5), 5% (v/v) methanol and 1.2 M ammonium sulfate. The supernatant from the ammonium sulfate fractionation was added to this column and eluted using linear gradients of 50 mM Tris (pH 7.5), 5% (v/v) methanol, with decreasing
15 (1.2 - 0.0) M ammonium sulfate and increasing (0-50) % (v/v) ethylene glycol at 1 ml/min.

The active peak was pooled, dialyzed into 50 mM Tris, 0.1 M NaCl, 0.01 M CaCl₂, 20% (v/v) ethylene glycol, and subjected to a second fractionation by lectin affinity
20 chromatography using a 40 ml Affi-Gel concanavalin A column run at 1 ml/min. The sample was eluted in a stepwise fashion in the same buffer with 0, 10, and 500 mM methyl α -mannopyranoside added successively. Fractions from each step of the gradient were pooled and tested for
25 their capacity to stimulate motility.

In the third purification step, the sample that eluted at 500 mM α -methyl-mannopyranoside was dialyzed into 10 mM Tris (pH 7.5) with 30% (v/v) ethylene glycol and fractionated by weak anion exchange chromatography.
30 Chromatography was carried out on a ZORBAX BioSeries-WAX column using a Shimadzu BioLiquid chromatograph and eluted with a linear gradient of (0.0 - 0.4 M) sodium chloride at 3 ml/min.

The active peak was pooled, dialyzed against 0.1 M
35 sodium phosphate (pH 7.2), 10% (v/v) methanol, and 10%

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° (v/v) ethylene glycol, and subjected to a fourth fractionation step on a series of Spherigel TSK columns (4000SW, 4000SW, 3000SW, 2000SW, in that order). This molecular sieve step was run using the Shimadzu BioLiquid chromatograph at 0.4 ml/min.

5 The active peak was pooled and dialyzed into 10 mM Tris (pH 7.5), 5% (v/v) methanol, 20% (v/v) ethylene glycol and subjected to a fifth (strong anion exchange) chromatography step, a Pro-Pac PA1 column run at 1 ml/min using a Dionex BioLC with AI450 software. The sample was
10 eluted with a linear gradient of (0.0-0.4M) NaCl.

In order to calculate activity yields after each step of purification, a unit of activity had to be derived. The dilution curve of ATX was biphasic with a broad peak and a linear range at sub-optimal concentrations. One unit
15 of activity/well (i.e., 40 units/ml) was defined as 50% of the maximal activity in a full dilution curve. This allowed calculation of the activity contained in any volume from the dilution needed to achieve 1 unit/well. Therefore, if a 1:10 dilution were needed in order to
20 produce 1 unit of activity/well, the material contained $10 \times 40 = 400$ units/ml.

Gel Electrophoresis. Protein samples were analyzed by SDS-polyacrylamide gel electrophoresis using the conditions of Laemmli (Laemmli, 1970). In brief, 7 or 8% SDS-containing
25 polyacrylamide gels were prepared or pre-poured (8-16%) gradient gels were obtained commercially. Samples were prepared with or without reducing conditions (5% β -mercaptoethanol). After electrophoretic separation, the gels were stained using Coomassie Blue G-250 as previously
30 described (Neuhoff, et al., 1988). In this staining protocol, which ordinarily requires no destaining step, the Coomassie stain appears to be able to stain as little as 10 ng of protein.

For two-dimensional electrophoresis, the protein, in
35 20% ethylene glycol, was dried in a Speed-vac and

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° redissolved in loading solution: 9M urea, 1% (v/v) pH 3-10 Bio-Lyte, and 2.5% (v/v) Nonidet-P40. This sample was then subjected to isoelectric focusing (O'Farrell, 1975) using a Bio-Rad tube cell in 120 x 3 mm polyacrylamide tube gels containing 9M urea, 2% (v/v) pH 3-10 Bio-Lyte, 0.25% (v/v) pH 8-10 Bio-Lyte and 2.5% (v/v) Nonidet-P40. Reservoir solutions were 0.01 M phosphoric acid and 0.02 M NaOH. Non-equilibrium isoelectric focusing (O'Farrell, et al., 1977) was run initially with constant voltage (500 v) for 5 hr. Since the protein was basic, the procedure was repeated under equilibrium conditions (500 v for 17 hr.). Electrophoresis in the second dimension was performed on a 7.5% polyacrylamide using the conditions of Laemmli (1970). The gel was stained with Coomassie Blue G-250 as above.

15 Preparation of peptides for internal sequence of autotaxin. Homogeneous ATX was sequentially digested with cyanogen bromide and, following reduction and pyridylethylation, with trypsin (Stone, et al., 1989). The resulting fragments were then separated by gradient elution on an Aquapore RP300 C-8 reverse phase column: 0.1% (v/v) trifluoroacetic acid and (0-70)% acetonitrile over 85 min. at a flow rate of 0.2 ml/min. A Dionex AI450 BioLC system was utilized and fractions were collected manually while monitoring the absorbance at 215 nm.

25 Sequence analysis of peptides. The amino acid sequences of peptides resulting from digestion and purification of ATX peptides #1-7 and 12-18, corresponding to SEQ ID NO:1 through SEQ ID NO:7 and SEQ ID NO:26 through SEQ ID NO:32, respectively, were determined on a Porton Instruments 2020 off-line sequenator using standard program #1.

30 Phenylthiohydantoin amino acid analysis of sequenator runs were performed on a Beckman System Gold HPLC using a modified sodium acetate gradient program and a Hewlett-Packard C-18 column. ATX-100 (SEQ ID NO:8), ATX-101 (SEQ ID NO:9), ATX-102 (SEQ ID NO:10), ATX-103 (SEQ ID NO:11)

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° and ATX 104 (SEQ ID NO:33) were sequenced from gel-purified ATX.

Protein databases (Pearson, et al. 1988) that were searched for homologies in amino acid sequence with the ATX peptides include: GenBank (68.0), EMBL (27.0), SWISS-PROT (18.0), and GenPept (64.3).

EXAMPLE 1

Purification of Autotaxin

The A2058 cells had been previously shown to produce protein factors which stimulate motility in an autocrine fashion (Liotta, et al., 1986). Conditioned medium from these cells was therefore used to identify and purify a new motility-stimulating factor, which is here named autotaxin and referred to as ATX. Since the purification was monitored with a biological assay, motility-stimulating activity had to be maintained throughout. The activity proved to be labile to freezing, acidic buffers, proteases (but not DNase or RNase), reduction, strong chaotrophic agents (e.g. > 4 M urea), and a variety of organic solvents (isopropanol, ethanol, acetonitrile). An organic solvent, ethylene glycol, which did not decrease bioactivity, was added for both storage and chromatographic separation.

100-200 L of serum-free conditioned medium were required in order to produce enough ATX for amino acid sequence analysis. The medium contained low concentrations of both BSA (5 µg/ml) which was needed as a carrier protein and insulin (10 µg/ml) which was required to support cell growth in low protein medium. Ultrafiltration to concentrate this large volume was performed with low protein-binding YM30 membranes which retain molecular species with $M_r > 30,000$. As seen in Table 1, 200 L of conditioned medium prepared in this manner resulted in 10×10^6 units of activity. However, the initial unfractionated conditioned medium contained additional substances known to stimulate activity,

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° particularly insulin, which does not completely wash out in the ultrafiltration step and which is additive to the motility stimulating activity in a complex manner (Stracke, et al., 1989). This had to be taken into account in order to determine yields for subsequent steps in which insulin had been removed.

TABLE 1. PURIFICATION OF AUTOTAXIN

10	Purification Step Recovery	Protein (mg)	Activity ^a (total units)	Specific	
				Activity (units/mg)	(%) ^b
	200 L Conditioned Medium	33,000	10,000,000 ^c	300	
	Phenyl Sepharose	1,235	460,000	370	100
15	Concanavalin A	58	660,000	11,400	100
	Weak Anion Exchange	4.5	490,000	110,000	100
	TSK Molecular Sieves	~0.4 ^d	220,000	550,000	48
20	Strong Anion Exchange	~0.04 ^d	24,000 ^e	600,000	5.2

^a Activity calculated from Boyden chamber assay. The dilution which resulted in 50% of maximal activity (generally approximately 20 laser density units or ~40 cells/HPF) was chosen to have 1 unit of activity per well (equivalent to 40 units/ml).

25 ^b Recovery was estimated from activity, after the first purification column (i.e., phenyl sepharose).

^c Initial activity in the unfractionated conditioned medium reflected the fact that insulin was used in the medium as a necessary growth factor under low protein conditions.

^d Estimated protein is based on quantification by amino acid analysis.

30 ^e This specific activity for purified protein corresponds to ~10 fmol ATX/unit of motility activity (in a Boyden chamber well).

The first step in the purification involved fractionation by hydrophobic interaction chromatography using a phenyl Sepharose CL-4B column. The results are

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° shown in Figure 1. Most proteins, including insulin, eluted from the column in early fractions or in the void. However, the peak of activity eluted relatively late. The activity which was purified was estimated as 460,000 units \pm 20% (Table 1). As the pooled peak of activity from the phenyl Sepharose fractionation is considered to be the first sample without significant insulin contamination, subsequent yields are measured against its total activity. Gel electrophoresis of a small portion of the pooled peak of activity (Figure 6A, column 2) revealed a large number of protein bands with BSA predominant from the original conditioned medium.

In the second step of purification, the active peak was applied to the lectin affinity column, Affi-Gel concanavalin A. As shown in Figure 2, most protein (estimated to be 90% of the total absorbance at 280 nm) failed to bind to the column at all. The non-binding fraction contained essentially no motility-stimulating activity (see dotted line in Figure 2). When a linear gradient of methyl α -D-mannopyranoside was applied to the column, chemotactic activity eluted off in a prolonged zone, beginning at a concentration of approximately 20 mM sugar. Consequently, a step gradient was used to elute. Pure BSA failed to bind to con A.

Activity was found primarily in the 500 mM step of methyl α -D-mannopyranoside. There appeared to be no significant loss of activity as seen in Table 1; however, specific activity (activity/mg total protein) increased thirty-fold. Gel electrophoresis of the pooled and concentrated peak (Figure 6A, column 3) revealed that the BSA overload was no longer apparent and the number of bands were much reduced. When the unbound protein was concentrated and applied to a gel, it appeared identical to the active peak from phenyl Sepharose-4B with a large BSA band.

The third purification step involved

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fractionating the previous active peak by weak anion exchange chromatography as shown in Figure 3. Under the running conditions, activity eluted in a broad peak-shoulder or double peak in the middle of the shallow portion (0.0-0.4 M) of the NaCl gradient. The largest proportion of protein, lacking in motility-stimulating capacity, bound strongly to the column and eluted off in high salt (1 M NaCl). There appeared to be no significant loss of activity, though specific activity increased by twenty-fold (Table 1). Analysis by gel electrophoresis of both the peak (28-34 min. in Figure 3) and the shoulder (35-45 min. in Figure 3) is shown in Figure 6A (columns 4 and 5, respectively). Two predominant protein bands resulted: a broad doublet around 25-35 kDa and a second doublet around 110-130 kDa.

In the fourth purification step, the active peak was applied to a series of molecular sieves. Spectrophotometric monitoring of the eluant revealed two large peaks of protein (Figure 4). Activity corresponded to the first, higher molecular weight peak. Recovery of activity was ~48% with a five-fold increase in specific activity. Analysis by gel electrophoresis was performed under non-reducing and reducing conditions as shown in Figure 6B (columns 2 and 3, respectively). This fractionation step had essentially removed all contaminating protein of molecular weight < 55 kDa. The predominant band remaining has a molecular weight of 120 kDa unreduced and 125 kDa reduced; there are two minor bands with molecular weights 85 kDa and 60 kDa. The fact that the 120 kDa protein changes so little in electrophoretic mobility after reduction would tend to indicate a paucity of disulfide bonds. However, the existing disulfide bonds have functional significance because motility-stimulating activity is labile to reduction.

The fifth purification step involved

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fractionation of the active peak by strong anion exchange chromatography. As shown in Figure 5, activity corresponds to two broad optical absorbance peaks in the middle of the gradient with contaminating proteins eluting earlier. These two peaks were identical by amino acid analysis and by polyacrylamide gel electrophoretic separation. They presumably represent different glycosylation states of the same parent protein. Activity is shown in Figure 5 at two different sample dilutions. Several dilutions of the fractionated samples were often necessary in order to resolve the true "peak" of activity as the shape of the ATX dilution curve was not sharp due to saturation and down regulation at high concentrations. Recovery from this chromatographic step is lower (5% compared to phenyl Sepharose), as might be expected when a minute quantity of protein is applied to a column; however, specific activity again increased (Table 1). Analysis by gel electrophoresis revealed a single protein band at molecular weight 120 kDa, unreduced (Figure 6C, column 2).

EXAMPLE 2

Characterization of Autotaxin

Two dimensional gel electrophoresis of the purified protein (Figure 7) revealed a single predominant band. The band slopes downward slightly toward the basic side of the gel in a manner that is characteristic of glycosylated proteins. A basic pI of 7.7 ± 0.2 was essentially the same whether the isoelectric focusing was run under non-equilibrium conditions (5 hr.) or was allowed to go to equilibrium (17 hr.).

A dilution curve of the purified protein is shown in Figure 8. The protein is active in the picomolar range and 1 unit of activity appears to correspond to a concentration of 400-600 picomolar (or approximately 10 fmol of ATX/Boyden chamber well). When dilutions were begun at higher concentrations of ATX, the resultant curve showed a broad plateau with down-regulation at the highest

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concentrations. The motility response to purified autotaxin is highly sensitive to pertussis toxin (hereinafter referred to as "PT") (Table 2 and Figure 9) with approximately 95% inhibition of activity at 0.5 μ g/ml PT.

TABLE 2. Effect of Pertussis Toxin (PT) on Autotaxin-stimulated motility

	A2058 Motility Response (density units ¹)	
	control cells ²	Pertussis toxin-treated cells ³
Condition medium ⁴	60.3	0.4
Purified Autotaxin	38.5	0.0

¹ Chemotaxis quantitated by motility assay (Stracke, et al., 1978).

² A2058 cell suspended at 2×10^6 cells/ml in DMEM supplemented with 1 mg/ml bovine serum and rocked at room temperature for 1 hr.

³ As control with 0.5 μ g/ml pertussis toxin.

⁴ Prepared by adding DMEM without phenol red supplemented with 0.1 mg/ml bovine serum albumin to subconfluent flasks of A2058 cells. The medium was harvested after 2 days incubation at 37°C in a humidified atmosphere and concentrated 25-30 fold using an Amicon ultrafiltration assembly with a YM-30 membrane.

Checkerboard analysis was performed to assess the random (chemokinetic) versus the directed (chemotactic) nature of the motility response to ATX. Chambers were assembled with different concentrations of ATX above and below the filter, using ATX purified through the weak anion exchange fractionation step. Squares below the diagonal reflect response to a positive gradient, squares above reflect response to a negative gradient, and squares on the diagonal reflect random motility in the absence of a gradient. ATX stimulates both chemotactic and chemokinetic responses (Figure 10), with chemotactic responses as high as fifteen-fold above background and

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° chemokinesis as high as eight-fold above background.

Amino acid analysis after complete acid hydrolysis was used to quantitate purified protein. This hydrolysis was carried out on protein excised from a polyacrylamide gel and presumed to be pure. The analysis indicated that 2.7 nmol of protein was present after
5 fractionation on the molecular sieve. After fractionation by strong anion exchange chromatography, approximately 300 pmol remained. The results of the analysis are shown in Table 3.

10 TABLE 3. AMINO ACID COMPOSITION OF AUTOTAXIN
(CYS and TRP were not determined in this analysis)

	<u>Amino Acid</u>	<u>Residues/100</u>
	ASX	12.5
	THR	6.0
	SER	5.7
15	GLX	9.4
	PRO	7.4
	GLY	7.0
	ALA	3.9
	VAL	6.7
	MET	1.2
	ILE	4.3
20	LEU	9.0
	TYR	5.2
	PHE	5.2
	HIS	3.8
	LYS	7.4
	ARG	5.4

25

EXAMPLE 3

ATX Degradation and Determination of

Amino Acid Sequence

Attempts to obtain N-terminal sequence
30 information from purified ATX repeatedly proved futile. The purified protein was therefore, sequentially digested and the resulting peptides fractionated by reverse phase chromatography. The results are shown in Figure 11. Multiple sharp peaks including clusters at both the
35 hydrophilic and hydrophobic ends of the gradient are seen.

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Several of these peptide peaks were chosen randomly for Edman degradation and N-terminal amino acid sequence analysis. Seven of the eight peaks (shown in Figure 11) chosen gave clear single sequence information as seen in Table 4. Using material from a separate digestion and purification, the remaining four sequences were also obtained.

Separate sense and antisense oligonucleotide probes were synthesized according to the fragment sequences of Table 4 by methods known to one skilled in the art. Representative probes are shown in Table 5.

TABLE 4. Peptide sequences for Autotaxin.

	PEPTIDE NO.	AMINO ACID SEQUENCE	SEQ ID: NO:	NAME
15	1.	WHVA	SEQ ID NO:1	ATX 18
	2.	PLDVYK	SEQ ID NO:2	ATX 19
	3.	YPAFK	SEQ ID NO:3	ATX 20
	4.	QAEVS	SEQ ID NO:4	ATX 24
	5.	PEEVTRPNYL	SEQ ID NO:5	ATX 29
20	6.	YDVPWNETI	SEQ ID NO:6	ATX 47
	7.	VPPFENIELY	SEQ ID NO:7	ATX 48
	8.	GGQPLWITATK	SEQ ID NO:8	ATX 100
	9.	VNSMQTVFVGY- GPTFK	SEQ ID NO:9	ATX 101
25	10.	DIEHLTSLDFFR	SEQ ID NO:10	ATX 102
	11.	TEFLSNYLTNVDD- ITLVPETLGR	SEQ ID NO:11	ATX 103
	12.	QYLHQYGSS	SEQ ID NO:26	ATX 37
30	13.	VLNYF	SEQ ID NO:27	ATX 39
	14.	YLNAT	SEQ ID NO:28	ATX 40
	15.	HLLYGRPAVLY	SEQ ID NO:29	ATX 41
	16.	SYPEILTPADN	SEQ ID NO:30	ATX 44
	17.	XYGFLFPPYLSSSP	SEQ ID NO:31	ATX 53
35	18.	TFPNLYTFATGLY	SEQ ID NO:32	ATX 59

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19. VNVISGPIFDYDYDGLH SEQ ID NO:33 ATX 104
DTEDK

Peptide numbers 1-7 refer to peaks numbered in Figure 11. Peptide numbers 12-18 refer to peptides purified from the preparation which yielded peptide numbers 1-7. Peptides 8-11 and 19, are from a separate purification, not shown in Figure 11.

X refers to potentially glycosylated residues.

TABLE 5.

Oligonucleotides synthesized from peptide sequences of autotaxin (ATX). The number of the oligonucleotide corresponds to the ATX peptide number as per Table 4. The final letter suffix distinguishes whether the oligonucleotide is a sense (S) or antisense (A) sequence.

	<u>Oligo</u>	<u>Sequence</u>	<u>SEQ ID NO:</u>
15	A-18A	GTT-GGC-AGC-NAC-RTG-CCA	SEQ ID NO:12
	A-18S	TGG-CAY-GTN-GCT-GCC-AAC	SEQ ID NO:13
	A-20A	CTT-GAA-GGC-AGG-GTA	SEQ ID NO:14
	A-20S	TAY-CCT-GCN-TTY-AAG	SEQ ID NO:15
	A-29A	GGT-NAC-YTC-YTC-AGG	SEQ ID NO:16
20	A-29S	CCT-GAR-GAR-GTN-ACC	SEQ ID NO:17
	A-47A	NGT-NGC-RTC-RAA-TGG-CAC-RTC	SEQ ID NO:18
	A-47S	GAY-GTG-CCA-TTY-GAY-GCN-ACN	SEQ ID NO:19
	A-48A	GTT-DAT-RTT-STC-RAA-TGG-GGG	SEQ ID NO:20
	A-48S	CCC-CCA-TTT-GAG-AAC-ATC-AAC	SEQ ID NO:21
25	A-100A	CTT-NGT-NGC-NGT-DAT-CCA-NAR- GGG-YTG-GCC-GCC	SEQ ID NO:22
	A-100S	GGC-GGC-CAR-CCC-YTN-TGG-ATH- ACN-GCN-ACN-AAG	SEQ ID NO:23
30	A-101A	CTT-RAA-GGT-GGG-GCC-RTA-GCC- CAC-RAA-GAC-TGT-YTG-CAT	SEQ ID NO:24
	A-101S	ATG-CAR-ACA-GTC-TTY-GTG-GGC- TAY-GGC-CCC-ACC-TTY-AAR	SEQ ID NO:25

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EXAMPLE 4Antipeptide Antibodies

Rabbits were injected with ATX-101 (SEQ ID NO:10) which had been cross-linked to bovine serum albumin. Antisera from these rabbits was subjected to salt precipitation followed by purification using affinity chromatography with Affi-Gel 10 beads covalently linked to the peptide, ATX-101 (SEQ ID NO:10). This affinity purified antibody reacted with the partially purified protein on immunoblots. This same antibody has been used to perform immunohistochemical stains on human tissue.

EXAMPLE 5Enzymatic Deglycosylation of ATX

Purified ATX that was to be treated with peptide N-glycosidase F (PNGase F) was first dialyzed into 0.2 M sodium phosphate, 10% (v/v) ethylene glycol pH 7.0, using Centricon-30 ultrafiltration tubes. Varying concentrations of PNGase F were added to the ATX and incubated 16-18 hr. at 37°C. Complete digestion appeared to occur at concentrations of enzyme above 30 mU/ml (where 1 U converts 1mmol of substrate/min). For comparison, the experiments were repeated in the presence of 0.1 M β -mercaptoethanol or 0.1% (w/v) SDS plus 0.5% (v.v) Nonidet-P40. ATX that was to be treated with neuraminidase or O-glycosidase was dialyzed into 20 mM sodium phosphate, 0.1 M calcium acetate, and 10% (v/v) ethylene glycol (pH 7.2). Neuraminidase was added to a final concentration of 2 U/ml. For treatment with neuraminidase alone, this mixture was incubated 16-18 hr at 37°C. Since O-glycosidase requires the removal of terminal sialic acid residues for efficient deglycosylation, ATX was pre-incubated with neuraminidase for 30-125 mU/ml and incubated 16-18 hr. at 37°C. The treated ATX was then dialyzed into 50 mM Tris with 20% ethylene glycol for storage at 5°C.

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° Treatment of ATX with N-glycosylation altering agents

A2058 cells were split into four 150 cm² flasks and incubated until just subconfluent in DMEM supplemented with 10% fetal calf serum. The medium was then replaced with fresh 10% FCS/DMEM to which had been added DPBS for
5 control, 1mM dMAN, 1 mM NMdNM, or 10 mM (1.7 mg/ml) Swn. Concentrations of these pharmacological agents were similar to those previously described as inhibiting N-glycan processing enzymes in melanoma cells (Seftor, et al. 1991; Dennis, et al. 1990) as well as carcinoma cells
10 (Ogier, et al. 1990). On the next day, each flask was washed twice with Dulbecco's phosphate buffered saline with calcium ("DPBS") then 20 ml of Dulbecco's minimum essential medium ("DMEM") supplemented with 0.01% (w/v) bovine serum albumin ("BSA") was added. The same
15 concentration of each agent was added to the appropriate equilibrated flask and incubated for ~ 24 hr, after which the medium from each treatment group was collected, concentrated, washed into DPBS and stored at 5°C.

Cells from each flask were trypsinized and
20 counted. There was no loss of viability or reduced cell number in any of the treatment groups compared to control cells.

Effect of PNGase F on ATX

ATX binds to concanavalin A ("Con A") agarose
25 beads and is eluted with buffer containing 0.5 M methyl α-D-mannopyranoside, indicating that ATX is likely to contain mannose residues. Such mannose sugar residues are most characteristic of N-linked oligosaccharides. In order to verify that ATX contained asparagine-linked
30 oligosaccharides, we treated it with the endoglycosidase, PNGase F, which cleaves high mannose, hybrid, and complex N-linked oligosaccharides at the asparagine residue.

Partially purified ATX was treated with 60 mU/ml of enzyme under a variety of increasingly denaturing
35 conditions and then separated by polyacrylamide gel

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electrophoresis (Figure 16). Lane 1 shows untreated material; the 125 kDa band (arrow) is autotaxin. When this material is treated overnight with PNGase F under very mild conditions, the size of the 125 kDa band decreases to ~100- 105 kDa. Addition of 0.1 M b-mercaptoethanol (Lane 2) or 0.5% Nonidet-P40 (lane 3) to the ATX sample has no effect on the size of the resultant protein band. Even complete denaturation of ATX of boiling the sample for 3 min in 0.1% SDS with (lane 5) or without (lane 4) β -mercaptoethanol, followed by addition of 0.5% Nonidet-P40 to maintain enzymatic activity, has no effect on the final size of deglycosylated protein, indicating that the deglycosidation reaction was complete even under mild conditions.

Because these results showed that ATX contained N-linked oligosaccharide groups, it became important to see if these sugar moieties were necessary for stimulation of motility. The partially purified ATX sample was treated with varying concentrations of PNGase F (0.1 to 60 mU/ml) under mild, non-denaturing conditions. Analysis of the resulting digest by polyacrylamide gel electrophoresis is shown in Figure 17A. As this figure shows, the digestion was incomplete using from 0.1 to 10 mU/ml of enzyme and resulted in a smear of protein between 100-125 kDa. However, at higher concentrations of enzyme, cleavage of N-linked oligosaccharides from ATX appears to be complete. When these different digestion products were compared for their capacity to stimulate motility (Figure 17B), there was no significant difference between groups.

EXAMPLE 6

Cloning the 3' end of Autotaxin (4C11)

ATX is active in picomolar to nanomolar concentrations and is synthesized in very small concentrations by A2058 cells. As might be expected, the cDNA clone was relatively rare, requiring various strategies and multiple library screenings in order to

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° identify it (Figure 12). Attempts to utilize degenerate oligonucleotides deduced from known peptide sequences were unsuccessful--whether we used the oligo nucleotides for screening cDNA libraries or for reverse transcription of mRNA followed by amplification with the polymerase chain reaction (RT/PCR). We then utilized an affinity-purified anti-peptide ATX-102 antibodies to screen an A2058 expression library.

These anti-peptide antibodies were generated by methods well established in the art and described previously with slight modification (Wacher, et al., 1990). In brief, the previously identified peptide, ATX-102 (Stracke, et al., 1992), was synthesized on a Biosearch 9600 peptide synthesizer. It was then solubilized in 1X PBS containing 20% (v/v) DMSO and conjugated to the protein carrier, bovine serum albumin (BSA), with glutaraldehyde. For the first injection into New Zealand white rabbits, the BSA-peptide conjugate was emulsified with complete Freund's adjuvant and injected subcutaneously. For subsequent injections, the BSA-peptide conjugate was emulsified with incomplete Freund's adjuvant. The resultant antiserum was heat-inactivated at 56°C for 30 min. Immunoglobulins were precipitated out in 47% saturated ammonium sulfate, then redissolved and dialyzed into PBS. Antibodies were adsorbed onto peptide-conjugated Affi-Gel 10 resin (made using the BioRad protocol), eluted with 0.1 N acetic acid, and neutralized with 2 M Tris-HCl, pH 8. The resulting affinity-purified antibodies were dialyzed into DPBS, concentrated, aliquotted, and stored at -20°C. The antibodies were found to recognize a 125 kDa protein on immunoblots of partially purified A2058 conditioned medium and to preferentially stain some breast carcinoma cells compared to normal breast using immunohistochemical techniques.

An A2058 cDNA library was prepared by purifying poly-A purified mRNA from the cells then size-selecting

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° mRNA > 1000 bp for the preparation of cDNA. The cDNA
inserts were placed into λ gt11 directionally, using the
ProMega cDNA kit using standard methods well-established
in the field. LE 392 cells were infected with the λ gt11
and plaques were transferred onto nitrocellulose membranes
5 by overnight incubation at 37°C. The antibody was
incubated with the membranes in blocking buffer for 2 hr
at room temperature, using approximately twice the
concentration of antibody which gave a strong response on
Western blot analysis. Secondary antibody was goat anti-
10 rabbit immunoglobulin, and the blot was developed
colorimetrically with 4-chloro-1-naphthol.

Positive clones were confirmed by antibody
competition with specific peptides but not unrelated
peptides. Using this technique and multiple subclonings,
15 we obtained a partial cDNA clone of the autotaxin gene,
which we called 4C11. The 4C11 insert was removed from
 λ gt11 by restriction enzyme digests and subcloned into
pBluescript for sequencing by standard Sanger techniques
(Sanger, et al., 1977). The 4C11 clone contained bases,
20 including the poly-adenylated tail and the AATAAA
adenylation signal locus, i.e., it contained the 3'
terminus of the gene. It also included a 627 base open
reading frame. Database analysis of this nucleotide
sequence revealed that it is unique. The predicated amino
25 acid sequence for 4C11 is 209 amino acids long with exact
matches for 7 previously identified ATX peptides: (ATX-20,
ATX-34, ATX-102, ATX-104, ATX-204, ATX-215, and ATX-244).

EXAMPLE 7

Cloning the 5' terminus of ATX

30 Database analysis of the 3' terminus of the ATX
gene demonstrated a novel protein. However, we have found
an interesting homology that has helped to guide us in
exploring its function. ATX had a 45% amino acid identity
and a 57% nucleotide identity with PC-1, a marker of B
35 cell activation found on the surface of plasma cells.

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Using the PC-1 protein sequence as a guide, we found that ATX peptide homologies were scattered throughout the length of the protein. The only exception was the far amino terminus of PC-1, which includes the transmembrane and intracellular domains, and which had no homologies. Knowing approximate localization of the ATX peptides along the length of ATX, we then amplified different segments of ATX by the PCR (Figure 13). These amplified segments of DNA were then subcloned into plasmids utilizing the TA Cloning kit of ProMega. The PCR amplified DNA could then be sequenced using standard Sanger sequencing techniques (Sanger, et al., 1977).

Cloning of full length ATX gene

A reverse transcriptase reaction was performed using total or oligo-(dT) purified RNA from A2058 or N-tera 2D1 cells as template and an anti-sense primer from the 5' end of 4C11 (GCTCAGATAAGGAGGAAAGAG). This was followed by one or two PCR amplification of the resultant cDNA using the commercially available kit from Perkin-Elmer and following manufacturer's directions. These PCR reactions utilized nested antisense primers from 4C11 (GAATCCGTAGGACATCTGCTT and TGTAGGCCAAACAGTTCTGAC) as well as degenerate, nested sense primers deduced from ATX peptides: ATX-101 (AAYTCIATGACARACIGTITTYGTIG and TTYGTIGGITAYGGICCIACITTYAA), ATX-103 (AAYTAYCTIACIAAYGTIGAYGAYAT and GAYGAYATIACICTIGTICCGGIAC), or ATX-224 (TGYTTYGARYTICARGARGCIGGICCCIC). The amplified DNA was then purified from a polyacrylamide gel using standard procedures and ligated into the pCR™ plasmid using the TA cloning kit (Invitrogen Corporation) according to manufacturer's directions.

The 5' RACE kit was utilized to extend the 5' end of ATX cDNA using total RNA from N-tera 2D1 as template and previously obtained sequence as primer (GCTGTCTTCAAACACAGC). The 5' end of the A2058 synthesized

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- ° protein was obtained by using previously obtained sequence as primer (CTGGTGGCTGTAATCCATAGC) in a reverse transcriptase reaction with total A2058 RNA as template, followed by PCR amplification utilizing the 5' end of N-tera 2D1 sequence as sense primer
- 5 (CGTGAAGGCAAAGAGAACACG) and a nested antisense primer (GCTGTCTTCAAACACAGC). A2058 DNA encoding ATX is set forth in a SEQ ID NO:68 and the amino acid sequence is provided in SEQ ID NO:69.

DNA sequencing: DNA sequencing was performed using dideoxy methodology (Sanger, et al. 1977) and (³⁵S)dATP (Du Pont, New England Nuclear).

We have found one region between the 5' end of the 4C11 and the ATX peptide designated ATX-101, also referred to as the "hot spot". This region has been sequenced five times with different sequences found each time. The hot spot appears to be located within the region from approximately nucleotide 1670 to 1815. The consensus sequence is represented by amino acids position 559 through 604. Variations found include DNA sequence that results in single and multiple amino acid insertions. One sequence had a stop codon in this region and may have represented an intron. This region has been found to be variable in forms of ATX.

EXAMPLE 8

25 Cloning ATX in a human teratocarcinoma cell line

The fact that ATX is present in other cancer cells was confirmed by sequence information from N-tera 2D1, a human teratocarcinoma cell line. For these cells, a prepared cDNA library in λgt10 was amplified and the cDNA inserts were extracted. Using oligonucleotide primers based on known A2058 ATX sequence, DNA segments were amplified by PCR. The DNA segments were then subcloned into plasmids and sequenced as for A2058. We have 3104 bp DNA sequence for N-tera ATX (SEQ ID NO:66) and smaller portions thereof. This includes an open

- 40 -

- ° reading frame that codes for a putative protein containing 861 amino acids (SEQ ID NO:67) and smaller portions thereof. Like the A2058 ATX, the N-tera 2D1 sequence has homologies for multiple ATX peptides (Figure 15). Sequence homology between the A2058 and N-tera 2D1 cells is approximately 99%.

EXAMPLE 9Cloning 5' end of ATX in human normal liver

- The 5' end of ATX has proven difficult to obtain from either tumor cell line to date. Normal human liver mRNA was therefore amplified using the 5' RACE kit (Clontech) with known sequence from A2058 ATX as antisense primer. A DNA segment was obtained and has been sequenced. This segment codes for 979 amino acids, including an initiating methionine (SEQ ID NO:38). The putative protein sequence also includes a 20 amino acid transmembrane domain which is different from the tumor ATX's (SEQ ID NO:54), as shown in Table 7. Both tumorous forms of ATX apparently lack a transmembrane region and are instead secreted proteins.

Table 7

Nucleotide and Amino Acid Sequences Encoding Liver ATX Amino
Terminus containing the Transmembrane region

- 25 Protein Sequence (SEQ ID NO: 54)
Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Asp Ile Ser Leu Phe Thr Phe Ala Val Gly
Val Asn Ile Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala Glu Gly Trp
- 30 DNA Sequence (SEQ ID NO: 53)
ATGGCAAGGA GGAGCTCGTT CCAGTCGTGT CAAGATATAT CCCTGTTTAC
TTTTGCCGTT GGAGTCAATA TCTGCTTAGG ATTCACTGCA CATCGAATTA
AGAGAGCAGA AGGATGG

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EXAMPLE 10Domains of ATX

Searches of protein databases (Pearson, et. al. 1988) confirmed that the homology between ATX and PC-1 was present throughout the length of the extracellular portion of the molecules (Buckley, et. al., 1990; Funakoshi, et. al. 1992). There is a 45% amino acid identity and a 64% similarity between the 2 protein sequences (Fig. 18). For the cDNA sequence, the identity is ~57%.

These proteins share several interesting properties and domains (Fig. 19). Both have a number of potential N-linked glycosylation sites: four for ATX (Asn54, Asn463, Asn577, Asn859) and nine for PC-1. Both have adjacent somatomedin B domains near the amino end of the extracellular domain. This somatomedin B domain is a cysteine-rich region containing 3 presumed cystine cross-linkages. ATX has 33 Cys residues and PC-1 has 37; 30 of these Cys residues are identical in placement. Both proteins also contain the loop region of an EF hand (Buckley, et. al. 1990; Kretsinger, 1987). In addition, both proteins have a transmembrane/signal peptide region with a short intracellular peptide, common in ectoenzymes (Maroux, 1987). However, the amino acid identity between ATX and PC-1 in the intracellular and transmembrane regions is only 11%.

Finally, both proteins have a region homologous to the bovine intestinal phosphodiesterase enzymatic domain with conservation of the threonine that is thought to act as the intermediate phosphate binding site (Culp, et al. 1985). PC-1 has been demonstrated to have phosphodiesterase type I, nucleotide pyrophosphatase, and threonine-specific kinase enzymatic activities (Rebbe, et al. 1991; Oda, et al. 1991). In order to test whether purified ATX had type I phosphodiesterase activity, samples were incubated with p-nitrophenyl thymidine-5'-monophosphate at pH 8.9 for 30 min. Samples were assayed

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- ° in a 100 μ l volume containing 50 mM Tris-HCl, pH 8.9 and 5 mM p-nitrophenyl thymidine-5'-monophosphate. After a 30 minute incubation at 37 °C the reactions were terminated by addition of 900 μ l 0.1 N NaOH and the amount of product formed was determined by reading the absorbance at 410 nm.
- 5 ATX was found to hydrolyze the p-nitrophenyl thymidine-5'-monophosphate (Razzell, 1963) at a rate of 10 pmol/ng/min, a reaction rate similar to that reported for PC-1 (Oda, et al. 1993).

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All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

- 15 While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true
- 20 scope of the present invention and appended claims.

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30

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: STRACKE, MARY; LIOTTA, LANCE;
SCHIFFMANN, ELLIOTT; KRUTZSCH,
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- 5 (ii) TITLE OF INVENTION: MOTILITY STIMULATING
PROTEIN USEFUL IN CANCER DIAGNOSIS AND
THERAPY
- (iii) NUMBER OF SEQUENCES: 69
- 10 (iv) CORRESPONDENCE ADDRESS:
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- 15 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy Disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WordPerfect 5.1
- (vi) CURRENT APPLICATION DATA:
20 (A) APPLICATION NUMBER:
(B) FILING DATE: 24-MAY-1995
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER: 08/346,455
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- 25 (vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER: 08/249,182
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(A) APPLICATION NUMBER: 07/822,043
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- 48 -

°

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 5
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gln Ala Glu Val Ser
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(2) INFORMATION FOR SEQ ID NO:5:

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- 49 -

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
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- (2) INFORMATION FOR SEQ ID NO:6:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9
 10 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
- Tyr Asp Val Pro Trp Asn Glu Thr Ile
 1 5
- 15 (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
- Val Pro Pro Phe Glu Asn Ile Glu Leu Tyr
 1 5 10
- (2) INFORMATION FOR SEQ ID NO:8:
- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 11
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
- 30 Gly Gly Gln Pro Leu Trp Ile Thr Ala Thr Lys
 1 5 10
- (2) INFORMATION FOR SEQ ID NO:9:
- (i) SEQUENCE CHARACTERISTICS:
 35 (A) LENGTH: 16

- 50 -

- °
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Val Asn Ser Met Gln Thr Val Phe Val Gly Tyr Gly
 1 5 10

5
 Pro Thr Phe Lys
 15

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 12
 10 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Ile Glu His Leu Thr Ser Leu Asp Phe Phe Arg
 15 1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23
 (B) TYPE: amino acid
 20 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Thr Glu Phe Leu Ser Asn Tyr Leu Thr Asn Val Asp
 1 5 10

25 Asp Ile Thr Leu Val Pro Glu Thr Leu Gly Arg
 15 20

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18
 30 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

35 GTTGGCAGCN ACRTGCCA

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- (2) INFORMATION FOR SEQ ID NO:13:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 18
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 5 (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
- TGGCAYGTNG CTGCCAAC 18
- (2) INFORMATION FOR SEQ ID NO:14:
- 10 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
- 15 CTTGAAGGCA GGGTA 15
- (2) INFORMATION FOR SEQ ID NO:15:
- 20 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
- 25 TAYCCTGCNT TYAAG 15
- (2) INFORMATION FOR SEQ ID NO:16:
- 30 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
- 35 GGTNACYTCY TCAGG 15

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(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCTGARGARG TNACC

15

10 (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

NGTNGCRTCR AATGGCACRT C

21

20 (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAYGTGCCAT TYGAYGCNAC N

21

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

35 GTTDA TRTTS TCRAATGGGG G

21

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- 5 (2) INFORMATION FOR SEQ ID NO:21:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
- CCCCCATTTG AGAACATCAA C 21
- 10 (2) INFORMATION FOR SEQ ID NO:22:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 33
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
- CTTNGTNGCN GTDATCCANA RGGGYTGGCC GCC 33
- 20 (2) INFORMATION FOR SEQ ID NO:23:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 33
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
- GGCGGCCARC CCYTNTGGAT HACNGCNACN AAG 33
- (2) INFORMATION FOR SEQ ID NO:24:
- 30 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 39
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

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° (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTTRAAGGTG GGGCCRTAGC CCACRAAGAC TGTYTG CAT

39

(2) INFORMATION FOR SEQ ID NO:25:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATGCARACAG TCTTYGTGGG CTAYGGCCCC ACCTTYAAR

39

(2) INFORMATION FOR SEQ ID NO:26:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

20 Gln Tyr Leu His Gln Tyr Gly Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:27:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

30 Val Leu Asn Tyr Phe
1 5

(2) INFORMATION FOR SEQ ID NO:28:

35 (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 5
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Tyr Leu Asn Ala Thr
1 5

(2) INFORMATION FOR SEQ ID NO:29:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

His Leu Leu Tyr Gly Arg Pro Ala Val Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Tyr Pro Glu Ile Leu Thr Pro Ala Asp Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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° (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Xaa	Tyr	Gly	Phe	Leu	Phe	Pro	Pro	Tyr	Leu	Ser	Ser
1				5					10		
Ser	Pro										

5 (2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Thr	Phe	Pro	Asn	Leu	Tyr	Thr	Phe	Ala	Thr	Gly	Leu
1				5					10		
Tyr											

15

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Val	Asn	Val	Ile	Ser	Gly	Pro	Ile	Asp	Asp	Tyr	Asp
1				5					10		
Tyr	Asp	Gly	Leu	His	Asp	Thr	Glu	Asp	Lys		
		15				20					

25

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 829
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: Unknown

30

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: No

(vi) ORIGINAL SOURCE:

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- 5 (A) ORGANISM: Human
 (B) STRAIN:
 (C) INDIVIDUAL ISOLATE:
 (D) DEVELOPMENTAL STAGE:
 (E) HAPLOTYPE:
 (F) TISSUE TYPE:
 (G) CELL TYPE: Melanoma
 (H) CELL LINE: A2058
 (I) ORGANELLE:

- (ix) FEATURE:
 (A) NAME/KEY:
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 10 (D) OTHER INFORMATION: Putative protein
 sequence of A2058 Autotaxin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

	Cys	His	Asp	Phe	Asp	Glu	Leu	Cys	Leu	Lys	Thr	Ala
	1				5					10		
15	Arg	Gly	Trp	Glu	Cys	Thr	Lys	Asp	Arg	Cys	Gly	Glu
		15					20					
	Val	Arg	Asn	Glu	Glu	Asn	Ala	Cys	His	Cys	Ser	Glu
	25					30					35	
	Asp	Cys	Leu	Ala	Arg	Gly	Asp	Cys	Cys	Thr	Asn	Tyr
			40					45				
	Gln	Val	Val	Cys	Lys	Gly	Glu	Ser	His	Trp	Val	Asp
	50					55						60
20	Asp	Asp	Cys	Glu	Glu	Ile	Lys	Ala	Ala	Glu	Cys	Pro
					65					70		
	Ala	Gly	Phe	Val	Arg	Pro	Pro	Leu	Ile	Ile	Phe	Ser
			75				80					
	Val	Asp	Gly	Phe	Arg	Ala	Ser	Tyr	Met	Lys	Lys	Gly
	85				90						95	
	Ser	Lys	Val	Met	Pro	Asn	Ile	Glu	Lys	Leu	Arg	Ser
			100					105				
25	Cys	Gly	Thr	His	Ser	Pro	Tyr	Met	Arg	Pro	Val	Tyr
		110					115				120	
	Pro	Thr	Lys	Thr	Phe	Pro	Asn	Leu	Tyr	Thr	Leu	Ala
					125					130		
	Thr	Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Val	Gly
			135				140					
	Asn	Ser	Met	Tyr	Asp	Pro	Val	Phe	Asp	Ala	Thr	Phe
30		145			150					155		
	His	Leu	Arg	Gly	Arg	Glu	Lys	Phe	Asn	His	Arg	Trp
			160					165				
	Trp	Gly	Gly	Gln	Pro	Leu	Trp	Ile	Thr	Ala	Thr	Lys
		170					175				180	
	Gln	Gly	Val	Lys	Ala	Gly	Thr	Phe	Phe	Trp	Ser	Val
35					185					190		

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°	Val	Ile	Pro	His	Glu	Arg	Arg	Ile	Leu	Thr	Ile	Leu
			195					200				
	Arg	Trp	Leu	Thr	Leu	Pro	Asp	His	Glu	Arg	Pro	Ser
	205					210					215	
	Val	Tyr	Ala	Phe	Tyr	Ser	Glu	Gln	Pro	Asp	Phe	Ser
			220					225				
5	Gly	His	Lys	Tyr	Gly	Pro	Phe	Gly	Pro	Glu	Glu	Ser
		230					235					240
	Ser	Tyr	Gly	Ser	Pro	Phe	Thr	Pro	Ala	Lys	Arg	Pro
					245					250		
	Lys	Arg	Lys	Val	Ala	Pro	Lys	Arg	Arg	Gln	Glu	Arg
			255					260				
	Pro	Val	Ala	Pro	Pro	Lys	Lys	Arg	Arg	Arg	Lys	Ile
	265					270					275	
10	His	Arg	Met	Asp	His	Tyr	Ala	Ala	Glu	Thr	Arg	Gln
				280					285			
	Asp	Lys	Met	Thr	Asn	Pro	Leu	Arg	Glu	Ile	Asp	Lys
		290					295					300
	Ile	Val	Gly	Gln	Leu	Met	Asp	Gly	Leu	Lys	Gln	Leu
					305					310		
	Lys	Leu	Arg	Arg	Cys	Val	Asn	Val	Ile	Phe	Val	Gly
			315					320				
15	Asp	His	Gly	Met	Glu	Asp	Val	Thr	Cys	Asp	Arg	Thr
	325					330					335	
	Glu	Phe	Leu	Ser	Asn	Tyr	Leu	Thr	Asn	Val	Asp	Asp
				340					345			
	Ile	Thr	Leu	Val	Pro	Gly	Thr	Leu	Gly	Arg	Ile	Arg
		350					355					360
	Ser	Lys	Phe	Ser	Asn	Asn	Ala	Lys	Tyr	Asp	Pro	Lys
					365					370		
20	Ala	Ile	Ile	Ala	Asn	Leu	Thr	Cys	Lys	Lys	Pro	Asp
			375					380				
	Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	Gln	His	Leu	Pro
	385					390					395	
	Lys	Arg	Leu	His	Tyr	Ala	Asn	Asn	Arg	Arg	Ile	Glu
				400					405			
	Asp	Ile	His	Leu	Leu	Val	Glu	Arg	Arg	Trp	His	Val
		410					415					420
25	Ala	Arg	Lys	Pro	Leu	Asp	Val	Tyr	Lys	Lys	Pro	Ser
					425					430		
	Gly	Lys	Cys	Phe	Phe	Gln	Gly	Asp	His	Gly	Phe	Asp
			435					440				
	Asn	Lys	Val	Asn	Ser	Met	Gln	Thr	Val	Phe	Val	Gly
	445					450					455	
	Tyr	Gly	Pro	Thr	Phe	Lys	Tyr	Lys	Thr	Lys	Val	Pro
				460					465			
30	Pro	Phe	Glu	Asn	Ile	Glu	Leu	Tyr	Asn	Val	Met	Cys
		470					475					480
	Asp	Leu	Leu	Gly	Leu	Lys	Pro	Ala	Pro	Asn	Asn	Gly
					485					490		
	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Arg	Thr	Asn
			495					500				
35	Thr	Phe	Arg	Pro	Thr	Met	Pro	Glu	Glu	Val	Thr	Arg
	505					510					515	

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Pro Asn Tyr Pro Gly Ile Met Tyr Leu Gln Ser Asp
 520 525
 Asp Asp Leu Gly Cys Thr Cys Asp Asp Lys Val Glu
 530 535 540
 Pro Lys Asn Lys Leu Asp Glu Leu Asn Lys Arg Leu
 545 550
 5 His Thr Lys Gly Ser Thr Glu Glu Arg His Leu Leu
 555 560
 Tyr Gly Arg Pro Ala Val Leu Tyr Arg Thr Arg Tyr
 565 570 575
 Asp Ile Leu Tyr His Thr Asp Phe Glu Ser Gly Tyr
 580 585
 Ser Glu Ile Phe Leu Met Leu Leu Trp Thr Ser Tyr
 590 595 600
 10 Thr Val Ser Lys Gln Ala Glu Val Ser Ser Val Pro
 605 610
 Asp His Leu Thr Ser Cys Val Arg Pro Asp Val Arg
 615 620
 Val Ser Pro Ser Phe Ser Gln Asn Cys Leu Ala Tyr
 625 630 635
 Lys Asn Asp Lys Gln Met Ser Tyr Gly Phe Leu Phe
 640 645
 15 Pro Pro Tyr Leu Ser Ser Ser Pro Glu Ala Lys Tyr
 650 655 660
 Asp Ala Phe Leu Val Thr Asn Met Val Pro Met Tyr
 665 670
 Pro Ala Phe Lys Arg Val Trp Asn Tyr Phe Gln Arg
 675 680
 Val Leu Val Lys Lys Tyr Ala Ser Glu Arg Asn Gly
 685 690 695
 20 Val Asn Val Ile Ser Gly Pro Ile Phe Asp Tyr Asp
 700 705
 Tyr Asp Gly Leu His Asp Thr Glu Asp Lys Ile Lys
 710 715 720
 Gln Tyr Val Glu Gly Ser Ser Ile Pro Val Pro Thr
 725 730
 25 His Tyr Tyr Ser Ile Ile Thr Ser Cys Leu Asp Phe
 735 740
 Thr Gln Pro Ala Asp Lys Cys Asp Gly Pro Leu Ser
 745 750 755
 Val Ser Ser Phe Ile Leu Pro His Arg Pro Asp Asn
 760 765
 Glu Glu Ser Cys Asn Ser Ser Glu Asp Glu Ser Lys
 770 775 780
 30 Trp Val Glu Glu Leu Met Lys Met His Thr Ala Arg
 785 790
 Val Arg Asp Ile Glu His Leu Thr Ser Leu Asp Phe
 795 800
 Phe Arg Lys Thr Ser Arg Ser Tyr Pro Glu Ile Leu
 805 810 815
 35 Thr Leu Lys Thr Tyr Leu His Thr Tyr Glu Ser Glu
 820 825

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(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2946
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: Unknown
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Human
- (B) STRAIN:
- (C) INDIVIDUAL ISOLATE:
- (D) DEVELOPMENTAL STAGE:
- (E) HAPLOTYPE:
- (F) TISSUE TYPE:
- (G) CELL TYPE: Melanoma
- (H) CELL LINE: A2058
- (I) ORGANELLE:
- (ix) FEATURE:
- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Partial DNA Sequence
of A2058 Autotaxin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

	GCTGCCATGA	CTTTGATGAG	CTGTGTTTGA	AGACAGCCCCG	40
	TGGCTGGGAG	TGTACTAAGG	ACAGATGTGG	AGAAGTCAGA	80
25	AATGAAGAAA	ATGCCTGTCA	CTGCTCAGAG	GA CTGCTTGG	120
	CCAGGGGAGA	CTGCTGTACC	AATTACCAAG	TGGTTTGCAA	160
	AGGAGAGTCG	CATTGGGTTG	ATGATGACTG	TGAGGAAATA	200
	AAGGCCGCAG	AATGCCCTGC	AGGGTTTGTT	CGCCCTCCAT	240
	TAATCATCTT	CTCCGTGGAT	GGCTTCCGTG	CATCATA CAT	280
	GAAGAAAGGC	AGCAAAGTCA	TGCCTAATAT	TGAAAAACTA	320
	AGGTCTTGTG	GCACACACTC	TCCCTACATG	AGGCCGGTGT	360
30	ACCCAACTAA	AACCTTTCCT	AACTTATACA	CTTTGGCCAC	400
	TGGGCTATAT	CCAGAATCAC	ATGGAATTGT	TGGCAATTCA	440
	ATGTATGATC	CTGTATTTGA	TGCCACTTTT	CATCTGCGAG	480
	GGCGAGAGAA	ATTTAATCAT	AGATGGTGGG	GAGGTCAACC	520
	GCTATGGATT	ACAGCCACCA	AGCAAGGGGT	GAAAGCTGGA	560
	ACATTCTTTT	GGTCTGTTGT	CATCCCTCAC	GAGCGGAGAA	600
	TATTAACCAT	ATTGCGGTGG	CTCACCTGCG	CAGATCATGA	640
	GAGGCCTTCG	GTCTATGCCT	TCTATTCTGA	GCAACCTGAT	680
35	TTCTCTGGAC	ACAAATATGG	CCCTTTCGGC	CCTGAGGAGA	720

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	GTAGTTATGG	CTCACCTTTT	ACTCCGGCTA	AGAGACCTAA	760
	GAGGAAAGTT	GCCCCTAAGA	GGAGACAGGA	AAGACCAGTT	800
	GCTCCTCCAA	AGAAAAAGAAG	AAGAAAAATA	CATAGGATGG	840
	ATCATTATGC	TGCGGAAACT	CGTCAGGACA	AAATGACAAA	880
	TCCTCTGAGG	GAAATCGACA	AAATTGTGGG	GCAATTAATG	920
	GATGGACTGA	AACAACCTAAA	ACTGCGTCGG	TGTGTCAACG	960
5	TCATCTTTGT	CGGAGACCAT	GGAATGGAAG	ATGTCACATG	1000
	TGATAGAACT	GAGTTCTTGA	GTAATTACCT	AACTAATGTG	1040
	GATGATATTA	CTTTAGTGCC	TGGAACCTCTA	GGAAGAATTC	1080
	GATCCAAATT	TAGCAACAAT	GCTAAATATG	ACCCCAAAGC	1120
	CATTATTGCC	AATCTCACGT	GTAAAAAACC	AGATCAGCAC	1160
	TTTAAGCCTT	ACTTGAAACA	GCACCTTCCC	AAACGTTTGC	1200
	ACTATGCCAA	CAACAGAAGA	ATTGAGGATA	TCCATTTATT	1240
	GGTGGAAACGC	AGATGGCATG	TTGCAAGGAA	ACCTTTGGAT	1280
10	GTTTATAAGA	AACCATCAGG	AAAATGCTTT	TTCCAGGGAG	1320
	ACCACGGATT	TGATAACAAG	GTCAACAGCA	TGCAGACTGT	1360
	TTTTGTAGGT	TATGGCCCAA	CATTTAAGTA	CAAGACTAAA	1400
	GTGCCTCCAT	TTGAAAACAT	TGAACCTTAC	AATGTTATGT	1440
	GTGATCTCCT	GGGATTGAAG	CCAGCTCCTA	ATAATGGGAC	1480
	CCATGGAAGT	TTGAATCATC	TCCTGCGCAC	TAATACCTTC	1520
	AGGCCAACCA	TGCCAGAGGA	AGTTACCAGA	CCCAATTATC	1560
15	CAGGGATTAT	GTACCTTCAG	TCTGATTTTG	ACCTGGGCTG	1600
	CACTTGTGAT	GATAAGGTAG	AGCCAAAGAA	CAAGTTGGAT	1640
	GAACTCAACA	AACGGCTTCA	TACAAAAGGG	TCTACAGAAG	1680
	AGAGACACCT	CCTCTATGGG	CGACCTGCAG	TGCTTTATCG	1720
	GACTAGATAT	GATATCTTAT	ATCACACTGA	CTTTGAAAGT	1760
	GGTTATATG	AAATATTCCCT	AATGCTACTC	TGGACATCAT	1800
	ATACTGTTTC	CAAACAGGCT	GAGGTTTCCA	GCGTTCCTGA	1840
	CCATCTGACC	AGTTGCGTCC	GGCCTGATGT	CCGTGTTTCT	1880
20	CCGAGTTTCA	GTCAGAACTG	TTTGGCCTAC	AAAAATGATA	1920
	AGCAGATGTC	CTACGGATTG	CTCTTTCTCT	CTTATCTGAG	1960
	CTCTTCACCA	GAGGCTAAAT	ATGATGCATT	CCTTGTAACC	2000
	AATATGGTTC	CAATGTATCC	TGCTTTCAAA	CGGGTCTGGA	2040
	ATTATTTCCA	AAGGGTATTG	GTGAAGAAAT	ATGCTTCGGA	2080
	AAGAAATGGA	GTTAACGTGA	TAAGTGGACC	AATCTTCGAC	2120
	TATGACTATG	ATGGCTTACA	TGACACAGAA	GACAAAATAA	2160
25	AACAGTACGT	GGAAGGCAGT	TCCATTCCCTG	TTCCAACCTCA	2200
	CTACTACAGC	ATCATCACCA	GCTGTCTGGA	TTTCACTCAG	2240
	CCTGCCGACA	AGTGTGACGG	CCCTCTCTCT	GTGTCCTCCT	2280
	TCATCCTGCC	TCACCGGCCT	GACAAAGAGG	AGAGCTGCAA	2320
	TAGCTCAGAG	GACGAATCAA	AATGGGTAGA	AGAACTCATG	2360
	AAGATGCACA	CAGCTAGGGT	GCGTGACATT	GAACATCTCA	2400
	CCAGCCTGGA	CTTCTTCCGA	AAGACCAGCC	GCAGCTACCC	2440
	AGAAATCCTG	ACACTCAAGA	CATACCTGCA	TACATATGAG	2480
30	AGCGAGATT	AACCTTCTGA	GCATCTGCAG	TACAGTCTTA	2520
	TCAACTGGTT	GTATATTTTT	ATATTGTTTT	TGTATTTATT	2560
	AATTTGAAAC	CAGGACATTA	AAAATGTTAG	TATTTTAAATC	2600
	CTGTACCAAA	TCTGACATAT	TATGCCTGAA	TGACTCCACT	2640
	GTTTTTCTCT	AATGCTTGAT	TTAGGTAGCC	TTGTGTTCTG	2680
	AGTAGAGCTT	GTAATAAATA	CTGCAGCTTG	AGAAAAAGTG	2720
	GAAGCTTCTA	AATGGTGCTG	CAGATTTGAT	ATTTGCATTG	2760
35	AGGAAATATT	AATTTTCCAA	TGCACAGTTG	CCACATTTAG	2800
	TCCTGTACTG	TATGGAAACA	CTGATTTTGT	AAAGTTGCCT	2840

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TTATTTGCTG TTAAGTGTGA ACTATGACAG ATATATTTAA 2880
 GCCTTATAAA CCAATCTTAA ACATAATAAA TCACACATTC 2920
 AGTTTAAAAA AAAAAAAAAA AAAAAA 2946

(2) INFORMATION FOR SEQ ID NO:36:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 788
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: Unknown
- (ii) MOLECULE TYPE: protein
- 10 (iii) HYPOTHETICAL: No
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Human
 (B) STRAIN:
 (C) INDIVIDUAL ISOLATE:
 (D) DEVELOPMENTAL STAGE:
 (E) HAPLOTYPE:
 15 (F) TISSUE TYPE:
 (G) CELL TYPE: teratocarcinoma
 (H) CELL LINE: N-tera 2D1
 (I) ORGANELLE:
- (ix) FEATURE:
 (A) NAME/KEY:
 20 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION: N-tera 2D1 putative
 ATX protein sequence
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:
- 25 Cys Asp Asn Leu Cys Lys Ser Tyr Thr Ser Cys Cys
 1 5 10
 His Asp Phe Asp Glu Leu Cys Leu Lys Thr Ala Arg
 15 20
 Ala Trp Glu Cys Thr Lys Asp Arg Cys Gly Glu Val
 25 30 35
 Arg Asn Glu Glu Asn Ala Cys His Cys Ser Glu Asp
 40 45
 30 Cys Leu Ala Arg Gly Asp Cys Cys Thr Asn Tyr Gln
 50 55 60
 Val Val Cys Lys Gly Glu Ser His Trp Val Asp Asp
 65 70
 Asp Cys Glu Glu Ile Lys Ala Ala Glu Cys Leu Gln
 75 80
 Val Asp Ser Pro Ser Ile Asn His Leu Leu Arg Gly
 85 90 95
- 35

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°	Trp	Leu	Pro	Met	Thr	Ser	Tyr	Met	Lys	Lys	Gly	Ser
				100					105			
	Lys	Val	Met	Pro	Asn	Ile	Glu	Lys	Leu	Arg	Ser	Cys
	110						115					120
	Gly	Thr	His	Ser	Pro	Tyr	Met	Arg	Pro	Val	Tyr	Pro
				125						130		
	Thr	Lys	Thr	Phe	Pro	Asn	Leu	Tyr	Thr	Leu	Ala	Thr
			135					140				
5	Gly	Leu	Tyr	Pro	Glu	Ser	His	Gly	Ile	Val	Gly	Asn
	145					150					155	
	Ser	Met	Tyr	Asp	Pro	Val	Phe	Asp	Ala	Thr	Phe	His
			160						165			
	Leu	Arg	Gly	Arg	Glu	Lys	Phe	Asn	His	Arg	Trp	Trp
	170						175					180
10	Ala	Gly	Gln	Pro	Leu	Trp	Ile	Thr	Ala	Thr	Lys	Gln
				185						190		
	Arg	Gly	Glu	Ser	Trp	Asn	Ile	Leu	Leu	Val	Cys	Cys
			195					200				
	His	Pro	Ser	Arg	Ala	Glu	Ile	Leu	Thr	Ile	Leu	Gln
	205					210					215	
	Trp	Leu	Thr	Leu	Pro	Asp	His	Glu	Arg	Pro	Ser	Val
			220						225			
15	Tyr	Ala	Phe	Tyr	Ser	Glu	Gln	Pro	Asp	Phe	Ser	Gly
	230					235						240
	His	Lys	His	Met	Pro	Phe	Gly	Pro	Glu	Met	Pro	Asn
				245						250		
	Pro	Leu	Arg	Glu	Met	His	Lys	Ile	Val	Gly	Gln	Leu
			255					260				
	Met	Asp	Gly	Leu	Lys	Gln	Leu	Lys	Leu	His	Arg	Cys
	265					270					275	
20	Val	Asn	Val	Ile	Phe	Val	Glu	Thr	Met	Asp	Gly	Arg
			280						285			
	Cys	His	Met	Tyr	Arg	Thr	Glu	Phe	Leu	Ser	Asn	Tyr
	290					295						300
	Leu	Thr	Asn	Val	Asp	Asp	Ile	Thr	Leu	Val	Pro	Gly
				305						310		
	Thr	Leu	Gly	Arg	Ile	Arg	Ser	Lys	Phe	Ser	Asn	Asn
		315						320				
25	Ala	Lys	Tyr	Asp	Pro	Lys	Ala	Ile	Ile	Ala	Asn	Leu
	325					330					335	
	Thr	Cys	Lys	Lys	Pro	Asp	Gln	His	Phe	Lys	Pro	Tyr
			340						345			
	Leu	Lys	Gln	His	Leu	Pro	Lys	Arg	Leu	His	Tyr	Ala
	350						355					360
	Asn	Asn	Arg	Arg	Ile	Glu	Asp	Ile	His	Leu	Leu	Val
				365						370		
30	Glu	Arg	Arg	Trp	His	Val	Ala	Arg	Lys	Pro	Leu	Asp
		375						380				
	Val	Tyr	Lys	Lys	Pro	Ser	Gly	Asn	Ala	Phe	Ser	Arg
	385					390					395	
	Glu	Thr	Thr	Ala	Phe	Asp	Asn	Lys	Val	Asn	Ser	Met
			400						405			
35	Gln	Thr	Val	Phe	Val	Gly	Tyr	Gly	Pro	Thr	Phe	Lys
	410					415						420

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Tyr Lys Thr Lys Val Pro Pro Phe Glu Asn Ile Glu
 425 430
 Leu Tyr Asn Val Met Cys Asp Leu Leu Gly Leu Lys
 435 440
 Pro Ala Pro Asn Asn Gly Thr His Phe Ser Leu Asn
 445 450 455
 5 His Leu Leu Arg Thr Asn Thr Phe Arg Pro Thr Met
 460 465
 Pro Glu Glu Val Thr Arg Pro Asn Tyr Pro Gly Ile
 470 475 480
 Met Tyr Leu Gln Ser Asp Phe Asp Leu Gly Cys Thr
 485 490
 Cys Asp Asp Lys Val Glu Pro Lys Asn Lys Leu Asp
 495 500
 10 Glu Leu Asn Lys Arg Leu His Thr Lys Gly Ser Thr
 505 510 515
 Glu Glu Arg His Leu Leu Tyr Gly Asp Arg Pro Ala
 520 525
 Val Leu Tyr Arg Thr Arg Tyr Asp Ile Leu Tyr His
 530 535 540
 Thr Asp Phe Glu Ser Gly Tyr Ser Glu Ile Phe Leu
 545 550
 15 Met Pro Leu Trp Thr Ser Tyr Thr Val Ser Lys Gln
 555 560
 Ala Glu Val Ser Ser Val Pro Asp His Leu Thr Ser
 565 570 575
 Cys Val Arg Pro Asp Val Arg Val Ser Pro Ser Phe
 580 585
 20 Ser Gln Asn Cys Leu Ala Tyr Lys Asn Asp Lys Gln
 590 595 600
 Met Ser Tyr Gly Gly Leu Gly Pro Pro Tyr Leu Ser
 605 610
 Ser Ser Pro Glu Ala Lys Tyr Asp Ala Phe Leu Val
 615 620
 Thr Asn Met Val Pro Met Tyr Pro Ala Phe Lys Arg
 625 630 635
 25 Val Trp Asn Tyr Phe Gln Arg Val Leu Val Lys Lys
 640 645
 Tyr Ala Ser Glu Arg Asn Gly Val Asn Val Ile Ser
 650 655 660
 Gly Pro Ile Phe Asp Tyr Asp Tyr Asp Gly Leu His
 665 670
 Asp Thr Glu Asp Lys Ile Lys Gln Tyr Val Glu Gly
 675 680
 30 Ser Ser Ile Pro Val Pro Thr His Tyr Tyr Ser Ile
 685 690 695
 Ile Thr Ser Cys Leu Asp Phe Thr Gln Pro Ala Asp
 700 705
 Lys Cys Asp Gly Pro Leu Ser Val Ser Ser Phe Ile
 710 715 720
 Leu Pro His Arg Pro Asp Asn Glu Glu Ser Cys Asn
 725 730
 35

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° Ser Ser Glu Asp Glu Ser Lys Trp Val Glu Glu Leu
 735 740
 Met Lys Met His Thr Ala Arg Val Arg Asp Ile Glu
 745 750 755
 His Leu Thr Ser Leu Asp Phe Phe Arg Lys Thr Ser
 760 765
 Arg Ser Tyr Pro Glu Ile Leu Thr Leu Lys Thr Tyr
 5 770 775 780
 Leu His Thr Tyr Glu Ser Glu Ile
 785

(2) INFORMATION FOR SEQ ID NO:37:

- 10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2712
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: Unknown

 (ii) MOLECULE TYPE: cDNA

 15 (iii) HYPOTHETICAL: No

 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Human
 (B) STRAIN:
 (C) INDIVIDUAL ISOLATE:
 (D) DEVELOPMENTAL STAGE:
 (E) HAPLOTYPE:
 20 (F) TISSUE TYPE:
 (G) CELL TYPE: teratocarcinoma
 (H) CELL LINE: N-tera 2D1
 (I) ORGANELLE:

 (ix) FEATURE:
 (A) NAME/KEY:
 25 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION: N-tera 2D1 ATX DNA
 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

	TGTGACA	ACT	TGTGTA	AAGAG	CTATAC	CAGT	TGCTGC	CCATG	40
30	ACTTTG	ATGA	GCTGTG	TTTG	AAGAC	AGCCC	GTGCGT	GCGGA	80
	GTGTAC	TAAG	GACAG	ATGTG	GGGA	AGTC	AG	AAATGA	120
	AATGC	CTGC	ACTG	CTCAG	GGACT	GCTTG	GCCAG	GGGAG	160
	ACTGC	TGTAA	CAATT	ACCAA	GTGGT	TTTG	CA	AAGG	200
	GCATT	G	GGTT	GATG	ATG	ACT	GTG	AGG	240
	AAATG	CCCTG	C	AGG	TTT	G	TTC	GCC	280
	CTCC	G	TGG	ATG	ACAT	C	ATAC	A	320
	CAGCA	AAG	TC	ATG	CC	TA	ATA	TTGA	360
35	GCAC	AC	ACT	CTCC	CT	AC	AT	GAGG	400

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	AAACCTTTCC	TAACTTATAC	ACTTTGGCCA	CTGGGCTATA	440
	TCCAGAATCA	CATGGAATTG	TTGGCAATTC	AATGTATGAT	480
	CCTGTATTTG	ATGCCACTTT	TCATCTGCGA	GGGCGAGAGA	520
	AATTTAATCA	TAGATGGTGG	GGAGGTCAAC	CGCTATGGAT	560
	TACAGCCACC	AAGCAAAGGG	GTGAAAGCTG	GAACATTCTT	600
	TTGGTCTGTT	GTCATCCCTC	ACGAGCGGAG	ATATTAACCA	640
5	TATTGCAGTG	GCTCACCCCTG	CCAGATCATG	AGAGGCCTTC	680
	GGTCTATGCC	TTCTATTCTG	AGCAACCTGA	TTTCTCTGGA	720
	CACAAACATA	TGCCTTTCGG	CCCTGAGATG	ACAAATCCTC	760
	TGAGGGAAAT	GCACAAAATT	GTGGGGCAAT	TAATGGATGG	800
	ACTGAAACAA	CTAAAACTGC	ATCGGTGTGT	CAACGTCATC	840
	TTTGTGCGAG	CCATGGATGG	AAGATGTCAC	ATGTATAGAA	880
	CTGAGTTCTT	GAGTAATTAC	CTAACTAATG	TGGATGATAT	920
	TACTTTAGTG	CCTGGAACCTC	TAGGAAGAAT	TCGATCCAAA	960
10	TTTAGCAACA	ATGCTAAATA	TCACCCCAAA	GCCATTATTG	1000
	CCAATCTCAC	GTGTAAAAAA	CCAGATCAGC	ACTTTAAGCC	1040
	TTACTTGAAA	CAGCACCTTC	CCAAACGTTT	GCACTATGCC	1080
	AACAACAGAA	GAATTGAGGA	TATCCATTTA	TTGGTGGAAC	1120
	GCAGATGGCA	TGTTGCAAGG	AAACCTTTGG	ATGTTTATAA	1160
	GAAACCATCA	GGAAATGCTT	TTTCCAGGGA	GACCACGGCA	1200
	TTTGATAACA	AGGTCAACAG	CATGCAGACT	GTTTTTGTAG	1240
15	GTTATGGCCC	AACATTTAAG	TACAAGACTA	AAGTDCCTCC	1280
	ATTTGAAAC	ATTGAACTTT	AAAATGTTAT	GTGTGATCTC	1320
	CTGGGATTGA	AGCCAGCTCC	TAATAATGGG	ACCCATGGAA	1360
	GTTTGAATCA	TCTCCTGCGC	ACTAATACCT	TCAGGCCAAC	1400
	CATGCCAGAG	GAAGTTACCA	GACCCTATTA	TCCAGGGATT	1440
	ATGTACCTTC	AGTCTGATTT	TGACCTGGGC	TGCACTTGTG	1480
	ATGATAAGGT	AGAGCCAAAG	AACAAGTTGG	ATGAACTCAA	1520
	CAACCGGCTT	CATACAAAAG	GGTCTACAGA	AGAGAGACAC	1560
20	CTCCTCTATG	GGGATCGACC	TGCAGTGCTT	TATCGGACTA	1600
	GATATGATAT	CTTATATCAC	ACTGACTTTG	AAAGTGGTTA	1640
	TAGTGAAATA	TTCTTAATGC	CACTCTGGAC	ATCATATACT	1680
	GTTTCCAAAC	AGGCTGAGGT	TTCCAGCGTT	CCTGACCATC	1720
	TGACCAGTTG	CGTCCGGCCT	GATGTCCGTG	TTTCTCCGAG	1760
	TTTCAGTCAG	AACGTGTTTG	CCTACAAAAA	TGATAAGCAG	1800
	ATGTCCTACG	GATTCCTCTT	TCCTCCTTAT	CTGAGCTCTT	1840
25	CACCAGAGGC	TAAATATGAT	GCATTCCTTG	TAACCAATAT	1880
	GGTTCCAATG	TATCCTGCTT	TCAAACGGGT	CTGGAATTAT	1920
	TTCCAAAGGG	TATTGGTGAA	GAAATATGCT	TCGGAAAGAA	1960
	ATGGAGTTAA	CGTGATAAGT	GGACCAATCT	TCGACTATGA	2000
	CTATGATGGC	TTACATGACA	CAGAAGACAA	AATAAAACAG	2040
	TACGTGGAAG	GCAGTTCCAT	TCCTGTTCCA	ACTCACTACT	2080
	ACAGCATCAT	CACCAGCTGT	CTGGATTTCA	CTCAGCCTGC	2120
	CGACAAGTGT	GACGGCCCTC	TCTCTGTGTC	CTCCTTCATC	2160
30	CTGCCTCACC	GGCCTGACAA	CGAGGAGAGC	TGCAATAGCT	2200
	CAGAGGACGA	ATCAAAATGG	GTAGAAGAAC	TCATGAAGAT	2240
	GCACACAGCT	AGGGTGCGTG	ACATTGAACA	TCTCACCAGC	2280
	CTGGACTTCT	TCCGAAAGAC	CAGCCGCAGC	TACCCAGAAA	2320
	TCCTGACACT	CAAGACATAC	CTGCATACAT	ATGAGAGCGA	2360
	GATTTAACTT	TCTGAGCATC	TGCAGTACAG	TCTTATCAAC	2400
	TGGTTGTATA	TTTTTTATATT	GTTTTTGTAT	TTATTAATTT	2440
	GAAACCAGGA	CATTAAAAAT	GTTAGTATTT	TAATCCTGTA	2480
35	CCAAATCTGA	CATATTATGC	CTGAATGACT	CCACTGTTTT	2520

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TCTCTAATGC TTGATTTAGG TAGCCTTGTG TTCTGAGTAG 2560
 AGCTTGTAAT AAATACTGCA GCTTGAGTTT TTAGTGGAAG 2600
 CTTCTAAATG GTGCTGCAGA TTTGATATTT GCATTGAGGA 2640
 AATATTAATT TTCCAATGCA CAGTTGCCAC ATTTAGTCCT 2680
 GTACTGTATG GAAACACTGA TTTTGTAAG TT 2712

5 (2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 979
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: Unknown
- 10 (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: No
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Human
 - (B) STRAIN:
 - 15 (C) INDIVIDUAL ISOLATE:
 - (D) DEVELOPMENTAL STAGE:
 - (E) HAPLOTYPE:
 - (F) TISSUE TYPE: Liver
 - (G) CELL TYPE:
 - (H) CELL LINE:
 - (I) ORGANELLE:
- 20 (ix) FEATURE:
- (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: putative autotaxin
protein sequence from human liver
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Asp
 1 5 10
 Ile Ser Leu Phe Thr Phe Ala Val Gly Val Asn Ile
 15 20
 Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala
 25 30 35
 Glu Gly Trp Glu Glu Gly Pro Pro Thr Val Leu Ser
 40 45
 Asp Ser Pro Trp Thr Asn Ile Ser Gly Ser Cys Lys
 50 55 60
 Gly Arg Cys Phe Glu Leu Gln Glu Ala Gly Pro Pro
 65 70
 Asp Cys Arg Cys Asp Asn Leu Cys Lys Ser Tyr Thr
 75 80

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Ser Cys Cys His Asp Phe Asp Glu Leu Cys Leu Lys
 85 90 95
 Thr Ala Arg Ala Trp Glu Cys Thr Lys Asp Arg Cys
 100 105
 Gly Glu Val Arg Asn Glu Glu Asn Ala Cys His Cys
 110 115 120
 5 Ser Glu Asp Cys Leu Ala Arg Gly Asp Cys Cys Thr
 125 130
 Asn Tyr Gln Val Val Cys Lys Gly Glu Ser His Trp
 135 140
 Val Asp Asp Asp Cys Glu Glu Ile Lys Ala Ala Glu
 145 150 155
 Cys Leu Gln Val Cys Ser Pro Ser Ile Asn His Leu
 160 165
 10 Leu Arg Gly Trp Leu Pro Met Thr Ser Tyr Met Lys
 170 175 180
 Lys Gly Ser Lys Val Met Pro Asn Ile Glu Lys Leu
 185 190
 Arg Ser Cys Gly Thr His Ser Pro Tyr Met Arg Pro
 195 200
 Val Tyr Pro Thr Lys Thr Phe Pro Asn Leu Tyr Thr
 205 210 215
 15 Leu Ala Thr Gly Leu Tyr Pro Glu Ser His Gly Ile
 220 225
 Val Gly Asn Ser Met Tyr Asp Pro Val Phe Asp Ala
 230 235 240
 Thr Phe His Leu Arg Gly Arg Glu Lys Phe Asn His
 245 250
 Arg Trp Trp Gly Gly Gln Pro Leu Trp Ile Thr Ala
 255 260
 20 Thr Lys Gln Arg Gly Glu Ser Trp Asn Ile Leu Leu
 265 270 275
 Val Cys Cys His Pro Ser Arg Ala Glu Ile Leu Thr
 280 285
 Ile Leu Gln Trp Leu Thr Leu Pro Asp His Glu Arg
 290 295 300
 25 Pro Ser Val Tyr Ala Phe Tyr Ser Glu Gln Pro Asp
 305 310
 Phe Ser Gly His Lys His Met Pro Phe Gly Pro Glu
 315 320
 Met Thr Asn Pro Leu Arg Glu Met His Lys Ile Val
 325 330 335
 Gly Gln Leu Met Asp Gly Leu Lys Gln Leu Lys Leu
 340 345
 30 His Arg Cys Val Asn Val Ile Phe Val Glu Thr Met
 350 355 360
 Asp Gly Arg Cys His Met Tyr Arg Thr Glu Phe Leu
 365 370
 Ser Asn Tyr Leu Thr Asn Val Asp Asp Ile Thr Leu
 375 380
 Val Pro Gly Thr Leu Gly Arg Ile Arg Ser Lys Phe
 385 390 395
 35

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0	Ser	Asn	Asn	Ala	Lys	Tyr	Asp	Pro	Lys	Ala	Ile	Ile
				400					405			
	Ala	Asn	Leu	Thr	Cys	Lys	Lys	Pro	Asp	Gln	His	Phe
		410					415					420
	Lys	Pro	Tyr	Leu	Lys	Gln	His	Leu	Pro	Lys	Arg	Leu
					425					430		
	His	Tyr	Ala	Asn	Asn	Arg	Arg	Ile	Glu	Asp	Ile	His
			435					440				
5	Leu	Leu	Val	Glu	Arg	Arg	Trp	His	Val	Ala	Arg	Lys
	445					450					455	
	Pro	Leu	Asp	Val	Tyr	Lys	Lys	Pro	Ser	Gly	Asn	Ala
				460					465			
	Phe	Ser	Arg	Glu	Thr	Thr	Ala	Phe	Asp	Asn	Lys	Val
		470					475					480
10	Asn	Ser	Met	Gln	Thr	Val	Phe	Val	Gly	Tyr	Gly	Pro
					485					490		
	Thr	Phe	Lys	Tyr	Lys	Thr	Lys	Val	Pro	Pro	Phe	Glu
			495					500				
	Asn	Ile	Glu	Leu	Tyr	Asn	Val	Met	Cys	Asp	Leu	Leu
	505					510					515	
	Gly	Leu	Lys	Pro	Ala	Pro	Asn	Asn	Gly	Thr	His	Gly
				520					525			
15	Ser	Leu	Asn	His	Leu	Leu	Arg	Thr	Asn	Thr	Phe	Arg
		530					535					540
	Pro	Thr	Met	Pro	Glu	Glu	Val	Thr	Arg	Pro	Asn	Tyr
					545					550		
	Pro	Gly	Ile	Met	Tyr	Leu	Gln	Ser	Asp	Phe	Asp	Leu
			555					560				
	Gly	Cys	Thr	Cys	Asp	Asp	Lys	Val	Glu	Pro	Lys	Asn
	565					570					575	
20	Lys	Leu	Asp	Glu	Leu	Asn	Lys	Arg	Leu	His	Thr	Lys
				580					585			
	Gly	Ser	Thr	Glu	Glu	Arg	His	Leu	Leu	Tyr	Gly	Asp
		590					595					600
	Arg	Pro	Ala	Val	Leu	Tyr	Arg	Thr	Arg	Tyr	Asp	Ile
					605					610		
	Leu	Tyr	His	Thr	Asp	Phe	Glu	Ser	Gly	Tyr	Ser	Glu
			615					620				
25	Ile	Phe	Leu	Met	Pro	Leu	Trp	Thr	Ser	Tyr	Thr	Val
	625					630					635	
	Ser	Lys	Gln	Ala	Glu	Val	Ser	Ser	Val	Pro	Asp	His
				640					645			
	Leu	Thr	Ser	Cys	Val	Arg	Pro	Asp	Val	Arg	Val	Ser
		650					655					660
	Pro	Ser	Phe	Ser	Gln	Asn	Cys	Leu	Ala	Tyr	Lys	Asn
					665					670		
30	Asp	Lys	Gln	Met	Ser	Tyr	Gly	Phe	Leu	Phe	Pro	Pro
			675					680				
	Tyr	Leu	Ser	Ser	Ser	Pro	Glu	Ala	Lys	Tyr	Asp	Ala
						690					695	
	Phe	Leu	Val	Thr	Asn	Met	Val	Pro	Met	Tyr	Pro	Ala
				700					705			
35	Phe	Lys	Arg	Val	Trp	Asn	Tyr	Phe	Gln	Arg	Val	Leu

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	Val	Lys	Lys	Tyr	Ala 725	Ser	Glu	Arg	Asn	Gly 730	Val	Asn
	Val	Ile	Ser 735	Gly	Pro	Ile	Phe	Asp 740	Tyr	Asp	Tyr	Asp
	Gly 745	Leu	His	Asp	Thr	Glu 750	Asp	Lys	Ile	Lys	Gln 755	Tyr
5	Val	Glu	Gly	Ser 760	Ser	Ile	Pro	Val	Pro 765	Thr	His	Tyr
	Tyr 770	Ser	Ile	Ile	Thr	Ser	Cys 775	Leu	Asp	Phe	Thr	Gln 780
	Pro	Ala	Asp	Lys	Cys 785	Asp	Gly	Pro	Leu	Ser 790	Val	Ser
	Ser	Phe	Ile	Leu	Pro	His	Arg	Pro 800	Asp	Asn	Glu	Glu
10	Ser 805	Cys	Asn	Ser	Ser	Glu 810	Asp	Glu	Ser	Lys	Trp 815	Val
	Glu	Glu	Leu	Met 820	Lys	Met	His	Thr	Ala 825	Arg	Val	Arg
	Asp 830	Ile	Glu	His	Leu	Thr	Ser 835	Leu	Asp	Phe	Phe	Arg 840
	Lys	Thr	Ser	Arg	Ser 845	Tyr	Pro	Glu	Ile	Leu	Thr	Leu
15	Lys	Thr	Tyr	Leu	His	Thr	Tyr	Glu 860	Ser	Glu	Ile	Xaa
	Leu 865	Ser	Glu	His	Leu	Gln 870	Tyr	Ser	Leu	Ile	Asn 875	Trp
	Leu	Tyr	Ile	Phe 880	Ile	Leu	Phe	Leu	Tyr 885	Leu	Leu	Ile
	Xaa	Asn	Gln	Asp	Ile	Lys	Asn 895	Val	Ser	Ile	Leu	Ile 900
20	Leu	Tyr	Gln	Ile	Xaa 905	His	Ile	Met	Pro	Glu 910	Xaa	Leu
	His	Cys	Phe	Ser	Leu	Met	Leu	Asp 920	Leu	Gly	Ser	Leu
	Val 925	Phe	Xaa	Val	Glu	Leu 930	Val	Ile	Asn	Thr	Ala 935	Ala
25	Xaa	Val	Phe	Ser 940	Gly	Ser	Phe	Xaa	Met 945	Val	Leu	Gln
	Ile 950	Xaa	Tyr	Leu	His	Xaa	Gly 955	Asn	Ile	Asn	Phe	Pro 960
	Met	His	Ser	Cys	His 965	Ile	Xaa	Ser	Cys	Thr 970	Val	Trp
	Lys	His	Xaa	Phe	Cys	Lys	Val					
				975								

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8
(B) TYPE: amino acids
(C) STRANDEDNESS: single

35

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- ° (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
(A) DESCRIPTION: peptide
- (iii) HYPOTHETICAL: No
- 5 (ix) FEATURE:
(A) NAME/KEY: ATX-204
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:
- 10 Met His Thr Ala Arg Val Arg Asp
5
- (2) INFORMATION FOR SEQ ID NO:40:
- (i) SEQUENCE CHARACTERISTICS:
15 (A) LENGTH: 8
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: No
- 20 (ix) FEATURE:
(A) NAME/KEY: ATX-205
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:
- Phe Ser Asn Asn Ala Lys Tyr Asp
5
- (2) INFORMATION FOR SEQ ID NO:41:
- 30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE:
35 (A) DESCRIPTION: Peptide

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°

(iii) HYPOTHETICAL: No

(ix) FEATURE:

(A) NAME/KEY: ATX-209

(B) LOCATION:

(C) IDENTIFICATION METHOD:

5

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Val Met Pro Asn Ile Glu Lys

5

10 (2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8

(B) TYPE: amino acids

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE:

(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:

(A) NAME/KEY: ATX-210

20

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

25

Thr Ala Arg Gly Trp Glu Cys Thr

5

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 11

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:

(A) DESCRIPTION: Peptide

35

(iii) HYPOTHETICAL: No

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°

- (ix) FEATURE:
(A) NAME/KEY: ATX-212
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

5

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Xaa Asp Ser Pro Trp Thr Xaa Ile Ser Gly Ser
5 10

10

- (2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15

- (ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

- (iii) HYPOTHETICAL: No

20

- (ix) FEATURE:
(A) NAME/KEY: ATX-214
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

25

Leu Arg Ser Cys Gly Thr His Ser Pro Tyr Met
5 10

- (2) INFORMATION FOR SEQ ID NO:45:

30

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

- (iii) HYPOTHETICAL: No

35

- (ix) FEATURE:

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- (A) NAME/KEY: ATX-215/34A
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

5 Thr Tyr Leu His Thr Tyr Glu Ser
5

(2) INFORMATION FOR SEQ ID NO:46:

- 10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

- 15 (iii) HYPOTHETICAL: No

- (ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Ala Ile Ile Ala Asn Leu Thr Cys Lys Lys Pro Asp Gln
5 10

25 (2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 30 (ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

- (iii) HYPOTHETICAL: No

- (ix) FEATURE:
(A) NAME/KEY: ATX-216
(B) LOCATION:
35 (C) IDENTIFICATION METHOD:

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(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Ile Val Gly Gln Leu Met Asp Gly
5

5

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:

- (A) NAME/KEY: ATX-218/44
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

20 Thr Ser Arg Ser Tyr Pro Glu Ile Leu
5

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE:

- (A) DESCRIPTION: Peptide

30

(iii) HYPOTHETICAL: No

(ix) FEATURE:

- (A) NAME/KEY: ATX-223B/24
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

35

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gln Ala Glu Val Ser Ser Val Pro Asp
5

5 (2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-224
(B) LOCATION:
15 (C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Cys Phe Glu Leu Gln Glu Ala Gly Pro Pro Asp Asp Cys
5 10

20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
25 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

30 (ix) FEATURE:
(A) NAME/KEY: ATX-229
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

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° Ser Tyr Thr Ser Cys Cys His Asp Phe Asp Glu Leu
5 10

(2) INFORMATION FOR SEQ ID NO:52:

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5          (1)  SEQUENCE CHARACTERISTICS:
                (A)  LENGTH: 16
                (B)  TYPE: amino acid
                (C)  STRANDEDNESS: single
                (D)  TOPOLOGY: linear

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(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

10 (iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: ATX-224/53
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gln Met Ser Tyr Gly Phe Leu Phe Pro Pro Tyr Leu
1 5 10
Ser Ser Ser Pro
15

20 (2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:
(A) DESCRIPTION: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE:

35 (vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE: Liver
35 (G) CELL TYPE:

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(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: 5' end of human liver
ATX gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ATGGCAAGGA	GGAGCTCGTT	CCAGTCGTGT	CAAGATATAT	40
CCCTGTTTAC	TTTTGCCGTT	GGAGTCAATA	TCTGCTTAGG	80
ATTCAGTGCA	CATCGAATTA	AGAGAGCAGA	AGGATGG	117

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: Unknown

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Peptide

(iii) HYPOTHETICAL: No

(v) FRAGMENT TYPE: N-terminal fragment

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE:
(D) DEVELOPMENTAL STAGE:
(E) HAPLOTYPE:
(F) TISSUE TYPE: Liver
(G) CELL TYPE:
(H) CELL LINE:
(I) ORGANELLE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: N-terminal region
including transmembrane domain of liver
ATX protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

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° Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Asp
 1 5 10
 Ile Ser Leu Phe Thr Phe Ala Val Gly Val Asn Ile
 15 20
 Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala
 25 30 35
 Glu Gly Trp

5

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
 (A) NAME/KEY:
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION: Primer from 5' end of 4C11
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

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GCTCAGATAA GGAGGAAAGA G

21

25 (2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
 (A) NAME/KEY:
 (B) LOCATION:

30

35

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°

- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Nested primers from 4C11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

5 GAATCCGTAG GACATCTGCT T 21

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21
 - (B) TYPE: amino acid
 - 10 (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: No
- 15 (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: Nested primers from 4C11

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:
TGTAGGCCAA ACAGTTCTGA C 21

(2) INFORMATION FOR SEQ ID NO:58:

- 25 (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- 30 (iii) HYPOTHETICAL: No
- (iv) ANTI-SENSE: No
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - 35 (C) IDENTIFICATION METHOD:

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(D) OTHER INFORMATION: Nested sense primer
deduced from ATX-101, wherein N is
inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

5 AAYTCNATGC ARACNGTNTT YGTNG 25

(2) INFORMATION FOR SEQ ID NO:59:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

15 (iv) ANTI-SENSE: No

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION: Nested primer of ATX
-101, wherein N is inosine

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TTYGTNGGNT AYGGNCCNAC NTTYAA 26

(2) INFORMATION FOR SEQ ID NO:60:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30 (iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
35 (C) IDENTIFICATION METHOD:

- 82 -

(D) OTHER INFORMATION: Nested primer deduced
from ATX-103, wherein N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

AAYTAYCTNA CNAAYGTNGA YGAYAT

26

5

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

15

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Nested primer deduced
from ATX-103, wherein N is inosine

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GAYGAYATNA CNCTNGTNCC NNGNAC

26

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

30

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

35

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- ° (D) OTHER INFORMATION: Nested primer deduced from ATX-103, wherein N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TGYTTYGARY TNCARGARGC NGGNCCNCC

29

5

(2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

15

GCTGTCTTCA AACACAGC

18

(2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

25

CTGGTGGCTG TAATCCATAG C

21

(2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

35

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(iv) ANTI-SENSE: No

(ix) FEATURE:
 (A) NAME/KEY:
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 5 (D) OTHER INFORMATION: Primer for 5' end of
 N-tera 2D1 sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CGTGAAGGCA AAGAGAACAC G 21

10 (2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3104
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: Unknown

15 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(ix) FEATURE:
 (A) NAME/KEY: N-tera 2D1 ATX cDNA
 (B) LOCATION:
 20 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

AGTGC	ACTCC	GTGAAGGCAA	AGAGAACACG	CTGCAAAAGG	40
CTTTCC	AATA	ATCCTCGACA	TGGCAAGGAG	GAGCTCGTTC	80
CAGTCG	TGTC	AGATAATATC	CCTGTTC	ACT	120
GAGTCA	AATAT	CTGCTTAGGA	TTC	ACTGCAC	160
GAGAGC	AGAA	GGATGGGAGG	AAGGTCCTCC	TACAGTGCTA	200
TCAGACT	CCC	CCTGGACCAA	CATCTCCGGA	TCTTGCAAGG	240
GCAGGTG	GCTT	TGAACTTCAA	GAGGCTGGAC	CTCCTGATTG	280
TCGCTGTG	GAC	AACTTGTGTA	AGAGCTATAC	CAGTTGCTGC	320
CATGACT	TTG	ATGAGCTGTG	TTTGAAGACA	GCCCGTGCGT	360
GGGAGTGT	AC	TAAGGACAGA	TGTGGAGAAG	TCAGAAATGA	400
AGAAAATGCC		TGTCACTGCT	CAGAGGACTG	CTTGGCCAGG	440
GGAGACTG	GCT	GTACCAATTA	CCAAGTGGTT	TGCAAAGGAG	480
AGTCGC	ATTG	GGTTGATGAT	GACTGTGAGG	AAATAAAGGC	520
CGCAGA	ATGC	CCTGCAGGGT	TTGTTGCCCC	TCCATTAATC	560
ATCTTCT	CCG	TGGATGGCTT	CCGTGCATCA	TACATGAAGA	600
AAGGCAG	CAA	AGTCATGCCT	AATATTGAAA	AACTAAGGTC	640
TTGTGGC	ACA	CACTCGCCCC	ACATGAGGCC	GGTGTACCCA	680
35 ACTAAA	ACCT	TTCCTAACTT	ATACACTTTG	GCCACTGGGC	720

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	TATATCCAGA	ATCACATGGA	ATTGTTGGCA	ATTCAATGTA	760
	TGATCCTGTA	TTTGATGCCA	CTTTTCATCT	GCGAGGGCGA	800
	GAGAAATTTA	ATCATAGATG	GTGGGGAGGT	CAACCGCTAT	840
	GGATTACAGC	CACCAAGCAA	AGGGGTGAAA	GCTGGAACAT	880
	TCTTTTGGTC	TGTTGTCATC	CCTCACGAGC	GGAGATATTA	920
	ACCATATTGC	AGTGGCTCAC	CCTGCCAGAT	CATGAGAGGC	960
5	TTCGGTCTAT	GCCTTCTATT	CTGAGCAACC	TGATTTCTCT	1000
	GGACACAAAT	ATGCCTTTCG	GCCCTGAGAT	GACAAATCCT	1040
	CTGAGGGAAA	TCGACAAAAT	TGTGGGGCAA	TTAATGGATG	1080
	GACTGAAACA	ACTAAAAC TG	CATCGGTGTG	TCAACGTCAT	1120
	CTTTGTGCGA	GACCATGGAA	TGGAAGATGT	CACATGTGAT	1160
	AGAACTGAGT	TCTTGAGTAA	TTACCTAACT	AATGTGGATG	1200
	ATATTACTTT	AGTGCCTGGA	ACTCTAGGAA	TTGATCCAA	1240
	ATTTAGCAAC	AATGCTAAAT	ATGACCCCAA	AGCCATTATT	1280
10	GCCAATCTCA	CGTGTAATAA	ACCAGATCAG	CACFTTAAGC	1320
	CTTACTTGAA	ACAGCACCTT	CCCAAACGTT	TGCACTATGC	1360
	CAACAACAGA	AGAATTGAGG	ATATCCATTT	ATTGGTGGAA	1400
	CGCAGATGGC	ATGTTGCAAG	GAAACCTTTG	GATGTTTATA	1440
	AGAAACCATC	AGGAAAATGC	TTTTTCCAGG	GAGACCACGG	1480
	ATTTGATAAC	AAGGTCAACA	GCATGCAGAC	TGTTTTTGTA	1520
	GGTTATGGCC	CAACATTTAA	GTACAAGACT	AAAGTGCCTC	1560
15	CATTTGAAAA	CATTGAACTT	TACAATGTTA	TGTGTGATCT	1600
	CCTGGGATTG	AAGCCAGCTC	CTAATAATGG	GACCCATGGA	1640
	AGTTTGAATC	ATCTCCTGCG	CACTAATACC	TTCAGGCCAA	1680
	CCATGCCAGA	GGAAAGTTACC	AGACCCAATT	ATCCAGGGAT	1720
	TATGTACCTT	CAGTCTGATT	TTGACCTGGG	CTGCACTTGT	1760
	GATGATAAGG	TAGAGCCAAA	GAACAAGTTG	GATGAACTCA	1800
	ACAAACGGCT	TCATACAAAA	GGGTCTACAG	AAGAGAGACA	1840
	CCTCCTCTAT	GGGCGACCTG	CAGTGCTTTA	TCGGACTAGA	1880
20	TATGATGTCT	TATATCACAC	TGACTTTGAA	AGTGGTTATA	1920
	GTGAAATATT	CCTAATGCCA	CTCTGGACAT	CATATACTGT	1960
	TTCCAAACAG	GCTGAGGTTT	CCAGCGTTCC	TGACCATCTG	2000
	ACCAGTTGCG	TCCGGCCTGA	TGTCCGTGTT	TCTCCGAGTT	2040
	TCAGTCAGAA	CTGTTTGGCC	TACAAAAATG	ATAAGCAGAT	2080
	GTCCTACGGA	TTCTCTTTTC	CTCCTTATCT	GAGCTCTTCA	2120
	CCAGAGGCTA	AATATGATGC	ATTCTTTGTA	ACCAATATGG	2160
25	TTCCAATGTA	TCCTGCTTTT	AAACGGGTCT	GGAATTATTT	2200
	CCAAAGGGTA	TTGGTGAAGA	AATATGCTTC	GGAAAGAAAT	2240
	GGAGTTAACG	TGATAAGTGG	ACCAATCTTC	GACTATGACT	2280
	ATGATGGCTT	ACATGACACA	GAAGACAAAA	TAAAACAGTA	2320
	CGTGGAAGGC	AGTTCCATTC	CTGTTCCAAC	TCACTACTAC	2360
	AGCATCATCA	CCAGCTGTCT	GGATTTCAC T	CAGCCTGCCG	2400
	ACAAGTGTGA	CGGCCCTCTC	TCTGTGTCTT	CCTTCATCCT	2440
	CCGTACCCGG	CCTGACAACG	AGGAGAGCTG	CAATAGCTCA	2480
30	GAGGACGAAT	CAAAATGGGT	AGAAGAACTC	ATGAAGATGC	2520
	ACACGGCTAG	GGTGCGTGAC	ATTGAACATC	TCACCAGCCT	2560
	GGACTTCTTC	CGAAAGACCA	GCCGCAGCTA	CCCAGAAATC	2600
	CTGACACTCA	AGACATACCT	GCATACATAT	GAGAGCGAGA	2640
	TTTAACTTTC	TGAGCATCTG	CAGTACAGTC	TTATCAACTG	2680
	GTTGTATATT	TTTATATTGT	TTTTGTATTT	ATTAATTTGA	2720
	AACCAGGACA	TTAAAAATGT	TAGTATTTTA	ATCCTGTACC	2760
35	AAATCTGACA	TATTATGCCT	GAATGACTCC	ACTGTTTTTC	2800
	TCTAATGCTT	GATTTAGGTA	GCCTTGTGTT	CTGAGTAGAG	2840

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CTTGTAATAA ATACTGCAGC TTGAGTTTTT AGTGGAAGCT 2880
 TCTAAATGGT GCTGCAGATT TGATATTTGC ATTGAGGAAA 2920
 TATTAATTTT CCAATGCACA GTTGCCACAT TTAGTCCTGT 2960
 ACTGTATGGA AACACTGATT TTGTAAAGTT GCCTTTATTT 3000
 GCTGTTAACT GTTAACTATG ACAGATATAT TTAAGCCTTA 3040
 TAAACCAATC TTAAACATAA TAAATCACAC ATTCAGTTTT 3080
 5 TTCTGGTAAA AAAAAAAAAA AAAA 3104

(2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 861
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: Unknown

 (ii) MOLECULE TYPE: protein

 (iii) HYPOTHETICAL: No

 (ix) FEATURE:
 (A) NAME/KEY: N-tera 2D1 ATX protein
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

20 Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Ile
 1 5 10
 Ile Ser Leu Phe Thr Phe Ala Val Gly Val Asn Ile
 15 20
 Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala
 25 30 35
 25 Glu Gly Trp Glu Glu Gly Pro Pro Thr Val Leu Ser
 40 45
 Asp Ser Pro Trp Thr Asn Ile Ser Gly Ser Cys Lys
 50 55 60
 Gly Arg Cys Phe Glu Leu Gln Glu Ala Gly Pro Pro
 65 70
 Asp Cys Arg Cys Asp Asn Leu Cys Lys Ser Tyr Thr
 75 80
 30 Ser Cys Cys His Asp Phe Asp Glu Leu Cys Leu Lys
 85 90 95
 Thr Ala Arg Ala Trp Glu Cys Thr Lys Asp Arg Cys
 100 105
 Gly Glu Val Arg Asn Glu Glu Asn Ala Cys His Cys
 110 115 120
 Ser Glu Asp Cys Leu Ala Arg Gly Asp Cys Cys Thr
 125 130
 35

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° Asn Tyr Gln Val Val Cys Lys Gly Glu Ser His Trp
 135 140
 Val Asp Asp Asp Cys Glu Glu Ile Lys Ala Ala Glu
 145 150 155
 Cys Pro Ala Gly Phe Val Arg Pro Pro Leu Ile Ile
 160 165
 Phe Ser Val Asp Gly Phe Arg Ala Ser Tyr Met Lys
 170 175 180
 5 Lys Gly Ser Lys Val Met Pro Asn Ile Glu Lys Leu
 185 190
 Arg Ser Cys Gly Thr His Ser Pro His Met Arg Pro
 195 200
 Val Tyr Pro Thr Lys Thr Phe Pro Asn Leu Tyr Thr
 205 210 215
 10 Leu Ala Thr Gly Leu Tyr Pro Glu Ser His Gly Ile
 220 225
 Val Gly Asn Ser Met Tyr Asp Pro Val Phe Asp Ala
 230 235 240
 Thr Phe His Leu Arg Gly Arg Glu Lys Phe Asn His
 245 250
 Arg Trp Trp Gly Gly Gln Pro Leu Trp Ile Thr Ala
 255 260
 15 Thr Lys Gln Arg Gly Glu Ser Trp Asn Ile Leu Leu
 265 270 275
 Val Cys Cys His Pro Ser Arg Ala Glu Ile Leu Thr
 280 285
 Ile Leu Gln Trp Leu Thr Leu Pro Asp His Glu Arg
 290 295 300
 Leu Arg Ser Met Pro Ser Ile Leu Ser Asn Leu Ile
 305 310
 20 Ser Leu Asp Thr Asn Met Pro Phe Gly Pro Glu Met
 315 320
 Thr Asn Pro Leu Arg Glu Ile Asp Lys Ile Val Gly
 325 330 335
 Gln Leu Met Asp Gly Leu Lys Gln Leu Lys Leu His
 340 345
 Arg Cys Val Asn Val Ile Phe Val Gly Asp His Gly
 350 355 360
 25 Met Glu Asp Val Thr Cys Asp Arg Thr Glu Phe Leu
 365 370
 Ser Asn Tyr Leu Thr Asn Val Asp Asp Ile Thr Leu
 375 380
 Val Pro Gly Thr Leu Gly Ile Arg Ser Lys Phe Ser
 385 390 395
 Asn Asn Ala Lys Tyr Asp Pro Lys Ala Ile Ile Ala
 400 405
 30 Asn Leu Thr Cys Lys Lys Pro Asp Gln His Phe Lys
 410 415 420
 Pro Tyr Leu Lys Gln His Leu Pro Lys Arg Leu His
 425 430
 Tyr Ala Asn Asn Arg Arg Ile Glu Asp Ile His Leu
 435 440
 35 Leu Val Glu Arg Arg Trp His Val Ala Arg Lys Pro
 445 450 455

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Leu Asp Val Tyr Lys Lys Pro Ser Gly Lys Cys Phe
 460 465
 Phe Gln Gly Asp His Gly Phe Asp Asn Lys Val Asn
 470 475 480
 Ser Met Gln Thr Val Phe Val Gly Tyr Gly Pro Thr
 485 490
 5 Phe Lys Tyr Lys Thr Lys Val Pro Pro Phe Glu Asn
 495 500
 Ile Glu Leu Tyr Asn Val Met Cys Asp Leu Leu Gly
 505 510 515
 Leu Lys Pro Ala Pro Asn Asn Gly Thr His Gly Ser
 520 525
 Leu Asn His Leu Leu Arg Thr Asn Thr Phe Arg Pro
 530 535 540
 10 Thr Met Pro Glu Glu Val Thr Arg Pro Asn Tyr Pro
 545 550
 Gly Ile Met Tyr Leu Gln Ser Asp Phe Asp Leu Gly
 555 560
 Cys Thr Cys Asp Asp Lys Val Glu Pro Lys Asn Lys
 656 570 575
 Leu Asp Glu Leu Asn Lys Arg Leu His Thr Lys Gly
 580 585
 15 Ser Thr Glu Glu Arg His Leu Leu Tyr Gly Arg Pro
 590 595 600
 Ala Val Leu Tyr Arg Thr Arg Tyr Asp Val Leu Tyr
 605 610
 His Thr Asp Phe Glu Ser Gly Tyr Ser Glu Ile Phe
 615 620
 Leu Met Pro Leu Trp Thr Ser Tyr Thr Val Ser Lys
 625 630 635
 20 Gln Ala Glu Val Ser Ser Val Pro Asp His Leu Thr
 640 645
 Ser Cys Val Arg Pro Asp Val Arg Val Ser Pro Ser
 650 655 660
 Phe Ser Gln Asn Cys Leu Ala Tyr Lys Asn Asp Lys
 665 670
 25 Gln Met Ser Tyr Gly Phe Leu Phe Pro Pro Tyr Leu
 675 680
 Ser Ser Ser Pro Glu Ala Lys Tyr Asp Ala Phe Leu
 685 690 695
 Val Thr Asn Met Val Pro Met Tyr Pro Ala Phe Lys
 700 705
 Arg Val Trp Asn Tyr Phe Gln Arg Val Leu Val Lys
 710 715 720
 30 Lys Tyr Ala Ser Glu Arg Asn Gly Val Asn Val Ile
 725 730
 Ser Gly Pro Ile Phe Asp Tyr Asp Tyr Asp Gly Leu
 735 740
 His Asp Thr Glu Asp Lys Ile Lys Gln Tyr Val Glu
 745 750 755
 Gly Ser Ser Ile Pro Val Pro Thr His Tyr Tyr Ser
 760 765
 35

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° Ile Ile Thr Ser Cys Leu Asp Phe Thr Gln Pro Ala
 770 775 780
 Asp Lys Cys Asp Gly Pro Leu Ser Val Ser Ser Phe
 785 790
 Ile Leu Arg His Arg Pro Asp Asn Glu Glu Ser Cys
 795 800
 Asn Ser Ser Glu Asp Glu Ser Lys Trp Val Glu Glu
 5 805 810 815
 Leu Met Lys Met His Thr Ala Arg Val Arg Asp Ile
 820 825
 Glu His Leu Thr Ser Leu Asp Phe Phe Arg Lys Thr
 830 835 840
 Ser Arg Ser Tyr Pro Glu Ile Leu Thr Leu Lys Thr
 845 850
 10 Tyr Leu His Thr Tyr Glu Ser Glu Ile
 855 860

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3251
 (B) TYPE: nucleic acid
 15 (C) STRANDEDNESS: double
 (D) TOPOLOGY: Unknown

 (ii) MOLECULE TYPE: cDNA

 (iii) HYPOTHETICAL: No

 (ix) FEATURE:
 20 (A) NAME/KEY: A2058 ATX cDNA
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION:

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:
 25 CGTGAAGGCA AAGAGAACAC GCTGCAAAAG GCTTCCAAGA 40
 ATCCTCGACA TGGCAAGGAG GAGCTCGTTC CAGTCGTGTC 80
 AGATAATATC CCTGTTCAC TTTGCCGTTG GAGTCAGTAT 120
 CTGCTTAGGA TTTCACTGCAC ATCGAATTAA GAGAGCAGAA 160
 GGATGGGAGG AAGGTCCTCC TACAGTGCTA TCAGACTCCC 200
 CCTGGACCAA CATCTCCGGA TCTTGCAAGG GCAGGTGCTT 240
 TGAAC TTCAA GAGGCTGGAC CTCCTGATTG TCGCTGTGAC 280
 30 AACTTGTGTA AGAGCTATAC CAGTTGCTGC CATGACTTTG 320
 ATGAGCTGTG TTTGAAGACA GCCCGTGGCT GGGAGGTGAC 360
 TAAGGACAGA TGTGGAGAAG TCAGAAATGA AGAAAATGCC 400
 TGTCAC TGCT CAGAGGACTG CTTGGCCAGG GGAGACTGCT 440
 GTACCAATTA CCAAGTGGTT TGCAAAGGAG AGTCGCATTG 480
 GGT TGATGAT GACTGTGAGG AAATAAAGGC CGCAGAATGC 520
 CCTGCAGGGT TTGTTCCGCC TCCATTAATC ATCTTCTCCG 560
 TGGATGGCTT CCGTGCATCA TACATGAAGA AAGGCAGCAA 600
 35 AGTCATGCCT AATATTGAAA AACTAAGGTC TTGTGGCACA 640

- 90 -

0		CACTCTCCCT	ACATGAGGCC	GGTGTACCCA	ACTAAAACCT	680
		TTCCTAACTT	ATACACTTTG	GCCACTGGGC	TATATCCAGA	720
		ATCACATGGA	ATTGTTGGCA	ATTCAATGTA	TGATCCTGTA	760
		TTTGATGCCA	CTTTTCATCT	GCGAGGGCGA	GAGAAATTTA	800
		ATCATAGATG	GTGGGGAGGT	CAACCGCTAT	GGATTACAGC	840
		CACCAAGCAA	GGGGTGAAAG	CTGGAACATT	CTTTTGGTCT	880
5		GTTGTCATCC	CTCACGAGCG	GAGAATATTA	ACCATATTGC	920
		GGTGGCTCAC	CCTGCCAGAT	CATGAGAGGC	CTTCGGTCTA	960
		TGCCTTCTAT	TCTGAGCAAC	CTGATTTCTC	TGGACACAAA	1000
		TATGGCCCTT	TCGGCCCTGA	GGAGAGTAGT	TATGGCTCAC	1040
		CTTTTACTCC	GGCTAAGAGA	CCTAAGAGGA	AAGTTGCCCC	1080
		TAAGAGGAGA	CAGGAAAAGAC	CAGTTGCTCC	TCCAAAGAAA	1120
		AGAAGAAGAA	AAATACATAG	GATGGATCAT	TATGCTGCGG	1160
		AAACTCGTCA	GGACAAAATG	ACAAATCCTC	TGAGGGAAAT	1200
10		CGACAAAATT	GTGGGGCAAT	TAATGGATGG	ACTGAAACAA	1240
		CTAAAACCTG	GTCCGGTGTGT	CAACGTCATC	TTTGTCGGAG	1280
		ACCATGGAAT	GGAAGATGTC	ACATGTGATA	GAAGTGAGTT	1320
		CTTGAGTAAT	TACCTAACTA	ATGTGGATGA	TATTACTTTA	1360
		GTGCCTGGAA	CTCTAGGAAG	AATTCGATCC	AAATTTAGCA	1400
		ACAATGTATA	ATATGACCCC	AAAGCCATTA	TTGCCAATCT	1440
		CACGTGTAAA	AAACCAGATC	AGCACTTTAA	GCCTTACTTG	1480
15		AAACAGCACC	TTCCCAAACG	TTTGCCTAT	GCCAACAACA	1520
		GAAGAATTGA	GGATATCCAT	TTATTGGTGG	AACGCAGATG	1560
		GCATGTTGCA	AGGAAACCTT	TGGATGTTTA	TAAGAAACCA	1600
		TCAGGAAAAT	GCTTTTTCCT	GGGAGACCAC	GGATTTGATA	1640
		ACAAGGTCAA	CAGCATGCAG	ACTGTTTTTG	TAGGTTATGG	1680
		CCCAACATTT	AAGTACAAGA	CTAAAGTGCC	TCCATTTGAA	1720
		AACATTGAAC	TTTACAATGT	TATGTGTGAT	CTCCTGGGAT	1760
		TGAAGCCAGC	TCCTAATAAT	GGGACCCATG	GAAGTTTGAA	1800
20		TCATCTCCTG	CGCACTAATA	CCTTCAGGCC	AACCATGCCA	1840
		GAGGAAGTTA	CCAGACCCAA	TTATCCAGGG	ATTATGTACC	1880
		TTCAGTCTGA	TTTTGACCTG	GGCTGCACTT	GTGATGATAA	1920
		GGTAGAGCCA	AAGAACAAGT	TGGATGAACT	CAACAAACGG	1960
		CTTCATACAA	AAGGGTCTAC	AGAAGAGAGA	CACCTCCTCT	2000
		ATGGGCGACC	TGCAGTGCTT	TATCGGACTA	GATATGATAT	2040
		CTTATATCAC	ACTGACTTTG	AAAGTGGTTA	TAGTGAAATA	2080
25		TTCCTAATGC	TACTCTGGAC	ATCATATACT	GTTTCCAAAC	2120
		AGGCTGAGGT	TTCCAGCGTT	CCTGACCATC	TGACCAAGTTG	2160
		CGTCCGGCCT	GATGTCCGTG	TTTCTCCGAG	TTTCAGTCAG	2200
		AACTGTTTGG	CCTACAAAAA	TGATAAGCAG	ATGTCCTACG	2240
		GATTCTCTT	TCCTCCTTAT	CTGAGCTCTT	CACCAGAGGC	2280
		TAAATATGAT	GCATTCTTTG	TAACCAATAT	GGTTCCAATG	2320
		TATCCTGCTT	TCAAACGGGT	CTGGAATTAT	TTCCAAAGGG	2360
		TATTGGTGAA	GAAATATGCT	TCGGAAGAAA	ATGGAGTTAA	2400
30		CGTGATAAGT	GGACCAATCT	TCGACTATGA	CTATGATGGC	2440
		TTACATGACA	CAGAAGACAA	AATAAAACAG	TACGTGGAAG	2480
		GCAGTTCCAT	TCCTGTTCCA	ACTCACTACT	ACAGCATCAT	2520
		CACCAGCTGT	CTGGATTTCA	CTCAGCCTGC	CGACAAGTGT	2560
		GACGGCCCTC	TCTCTGTGTC	CTCCTTCATC	CTGCCTCACC	2600
		GGCCTGACAA	CGAGGAGAGC	TGCAATAGCT	CAGAGGACGA	2640
		ATCAAAATGG	GTAAGAAGAC	TCATGAAGAT	GCACACAGCT	2680
35		AGGGTGCGTG	ACATTGAACA	TCTCACCAGC	CTGGACTTCT	2720
		TCCGAAAGAC	CAGCCGCAGC	TACCCAGAAA	TCCTGACACT	2760

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CAAGACATAC CTGCATACAT ATGAGAGCGA GATTTAACTT 2800
 TCTGAGCATC TGCAGTACAG TCTTATCAAC TGGTTGTATA 2840
 TTTTATATTT GTTTTGTAT TTATTAATTT GAAACCAGGA 2880
 CATTAAAAAT GTTAGTATTT TAATCCTGTA CCAAATCTGA 2920
 CATATTATGC CTGAATGACT CCACTGTTTT TCTCTAATGC 2960
 TTGATTTAGG TAGCCTTGTG TTCTGAGTAG AGCTTGTAAT 3000
 5 AAATACTGCA GCTTGAGAAA AAGTGGAAGC TTCTAAATGG 3040
 TGCTGCAGAT TTGATATTTG CATTGAGGAA ATATTAATTT 3080
 TCCAATGCAC AGTTGCCACA TTTAGTCCTG TACTGTATGG 3120
 AAACACTGAT TTTGTAAAGT TGCCTTTATT TGCTGTAAAC 3160
 TGTTAACTAT GACAGATATA TTTAAGCCTT ATAAACCAAT 3200
 CTTAAACATA ATAAATCACA CATTCACTTT TAAAAAATAA 3240
 AAAAAAAAAA A 3251

10

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 915
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: Unknown

15

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: No

(ix) FEATURE:

- (A) NAME/KEY: A2058 ATX protein
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION:

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

25 Met Ala Arg Arg Ser Ser Phe Gln Ser Cys Gln Ile
 1 5 10
 Ile Ser Leu Phe Thr Phe Ala Val Gly Val Ser Ile
 15 20
 Cys Leu Gly Phe Thr Ala His Arg Ile Lys Arg Ala
 25 30 35
 Glu Gly Trp Glu Glu Gly Pro Pro Thr Val Leu Ser
 40 45
 30 Asp Ser Pro Trp Thr Asn Ile Ser Gly Ser Cys Lys
 50 55 60
 Gly Arg Cys Phe Glu Leu Gln Glu Ala Gly Pro Pro
 65 70
 Asp Cys Arg Cys Asp Asn Leu Cys Lys Ser Tyr Thr
 75 80
 Ser Cys Cys His Asp Phe Asp Glu Leu Cys Leu Lys
 85 90 95

35

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° Thr Ala Arg Gly Trp Glu Cys Thr Lys Asp Arg Cys
 100 105
 Gly Glu Val Arg Asn Glu Glu Asn Ala Cys His Cys
 110 115 120
 Ser Glu Asp Cys Leu Ala Arg Gly Asp Cys Cys Thr
 125 130
 Asn Tyr Gln Val Val Cys Lys Gly Glu Ser His Trp
 135 140
 5 Val Asp Asp Asp Cys Glu Glu Ile Lys Ala Ala Glu
 145 150 155
 Cys Pro Ala Gly Phe Val Arg Pro Pro Leu Ile Ile
 160 165
 Phe Ser Val Asp Gly Phe Arg Ala Ser Tyr Met Lys
 170 175 180
 10 Lys Gly Ser Lys Val Met Pro Asn Ile Glu Lys Leu
 185 190
 Arg Ser Cys Gly Thr His Ser Pro Tyr Met Arg Pro
 195 200
 Val Tyr Pro Thr Lys Thr Phe Pro Asn Leu Tyr Thr
 205 210 215
 Leu Ala Thr Gly Leu Tyr Pro Glu Ser His Gly Ile
 220 225
 15 Val Gly Asn Ser Met Tyr Asp Pro Val Phe Asp Ala
 230 235 240
 Thr Phe His Leu Arg Gly Arg Glu Lys Phe Asn His
 245 250
 Arg Trp Trp Gly Gly Gln Pro Leu Trp Ile Thr Ala
 255 260
 Thr Lys Gln Gly Val Lys Ala Gly Thr Phe Phe Trp
 265 270 275
 20 Ser Val Val Ile Pro His Glu Arg Arg Ile Leu Thr
 280 285
 Ile Leu Arg Trp Leu Thr Leu Pro Asp His Glu Arg
 290 295 300
 Pro Ser Val Tyr Ala Phe Tyr Ser Glu Gln Pro Asp
 305 310
 Phe Ser Gly His Lys Tyr Gly Pro Phe Gly Pro Glu
 315 320
 25 Glu Ser Ser Tyr Gly Ser Pro Phe Thr Pro Ala Lys
 325 330 335
 Arg Pro Lys Arg Lys Val Ala Pro Lys Arg Arg Gln
 340 345
 Glu Arg Pro Val Ala Pro Pro Lys Lys Arg Arg Arg
 350 355 360
 30 Lys Ile His Arg Met Asp His Tyr Ala Ala Glu Thr
 365 370
 Arg Gln Asp Lys Met Thr Asn Pro Leu Arg Glu Ile
 375 380
 Asp Lys Ile Val Gly Gln Leu Met Asp Gly Leu Lys
 385 390 395
 Gln Leu Lys Leu Arg Arg Cys Val Asn Val Ile Phe
 400 405
 35 Val Gly Asp His Gly Met Glu Asp Val Thr Cys Asp
 410 415 420

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	Arg	Thr	Glu	Phe	Leu	Ser	Asn	Tyr	Leu	Thr	Asn	Val
					425					430		
	Asp	Asp	Ile	Thr	Leu	Val	Pro	Gly	Thr	Leu	Gly	Arg
			435					440				
	Ile	Arg	Ser	Lys	Phe	Ser	Asn	Asn	Ala	Lys	Tyr	Asp
	445					450					455	
5	Pro	Lys	Ala	Ile	Ile	Ala	Asn	Leu	Thr	Cys	Lys	Lys
				460					465			
	Pro	Asp	Gln	His	Phe	Lys	Pro	Tyr	Leu	Lys	Gln	His
		470					475					480
	Leu	Pro	Lys	Arg	Leu	His	Tyr	Ala	Asn	Asn	Arg	Arg
					485					490		
	Ile	Glu	Asp	Ile	His	Leu	Leu	Val	Glu	Arg	Arg	Trp
			495					500				
10	His	Val	Ala	Arg	Lys	Pro	Leu	Asp	Val	Tyr	Lys	Lys
	505					510					515	
	Pro	Ser	Gly	Lys	Cys	Phe	Phe	Gln	Gly	Asp	His	Gly
				520					525			
	Phe	Asp	Asn	Lys	Val	Asn	Ser	Met	Gln	Thr	Val	Phe
		530					535					540
	Val	Gly	Tyr	Gly	Pro	Thr	Phe	Lys	Tyr	Lys	Thr	Lys
					545					550		
15	Val	Pro	Pro	Phe	Glu	Asn	Ile	Glu	Leu	Tyr	Asn	Val
				555				560				
	Met	Cys	Asp	Leu	Leu	Gly	Leu	Lys	Pro	Ala	Pro	Asn
	565					570					575	
	Asn	Gly	Thr	His	Gly	Ser	Leu	Asn	His	Leu	Leu	Arg
				580					585			
20	Thr	Asn	Thr	Phe	Arg	Pro	Thr	Met	Pro	Glu	Glu	Val
		590					595					600
	Thr	Arg	Pro	Asn	Tyr	Pro	Gly	Ile	Met	Tyr	Leu	Gln
					605					610		
	Ser	Asp	Phe	Asp	Leu	Gly	Cys	Thr	Cys	Asp	Asp	Lys
			615					620				
	Val	Glu	Pro	Lys	Asn	Lys	Leu	Asp	Glu	Leu	Asn	Lys
						630					635	
25	Arg	Leu	His	Thr	Lys	Gly	Ser	Thr	Glu	Glu	Arg	His
				640					645			
	Leu	Leu	Tyr	Gly	Arg	Pro	Ala	Val	Leu	Tyr	Arg	Thr
		650					655					660
	Arg	Tyr	Asp	Ile	Leu	Tyr	His	Thr	Asp	Phe	Glu	Ser
				665						670		
	Gly	Tyr	Ser	Glu	Ile	Phe	Leu	Met	Leu	Leu	Trp	Thr
			675					680				
30	Ser	Tyr	Thr	Val	Ser	Lys	Gln	Ala	Glu	Val	Ser	Ser
	685					690					695	
	Val	Pro	Asp	His	Leu	Thr	Ser	Cys	Val	Arg	Pro	Asp
				700					705			
	Val	Arg	Val	Ser	Pro	Ser	Phe	Ser	Gln	Asn	Cys	Leu
		710					715					720
	Ala	Tyr	Lys	Asn	Asp	Lys	Gln	Met	Ser	Tyr	Gly	Phe
					725					730		

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0	Leu	Phe	Pro	Pro	Tyr	Leu	Ser	Ser	Pro	Glu	Ala
			735					740			
	Lys	Tyr	Asp	Ala	Phe	Leu	Val	Thr	Asn	Met	Pro
	745					750				755	
	Met	Tyr	Pro	Ala	Phe	Lys	Arg	Val	Trp	Asn	Phe
				760				765			
	Gln	Arg	Val	Leu	Val	Lys	Lys	Tyr	Ala	Ser	Arg
	770					775					780
5	Asn	Gly	Val	Asn	Val	Ile	Ser	Gly	Pro	Ile	Asp
				785						790	
	Tyr	Asp	Tyr	Asp	Gly	Leu	His	Asp	Thr	Glu	Lys
			795					800			
	Ile	Lys	Gln	Tyr	Val	Glu	Gly	Ser	Ser	Ile	Val
	805					810				815	
10	Pro	Thr	His	Tyr	Tyr	Ser	Ile	Ile	Thr	Ser	Leu
				820					825		
	Asp	Phe	Thr	Gln	Pro	Ala	Asp	Lys	Cys	Asp	Pro
	830					835					840
	Leu	Ser	Val	Ser	Ser	Phe	Ile	Leu	Pro	His	Pro
				845						850	
	Asp	Asn	Glu	Glu	Ser	Cys	Asn	Ser	Ser	Glu	Glu
			855					860			
15	Ser	Lys	Trp	Val	Glu	Glu	Leu	Met	Lys	Met	Thr
	865					870				875	
	Ala	Arg	Val	Arg	Asp	Ile	Glu	His	Leu	Thr	Leu
				880					885		
	Asp	Phe	Phe	Arg	Lys	Thr	Ser	Arg	Ser	Tyr	Glu
	890					895					900
	Ile	Leu	Thr	Leu	Lys	Thr	Tyr	Leu	His	Thr	Glu
				905						910	
20	Ser	Glu	Ile								
			915								

25

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° CLAIMS:

1. A DNA segment coding for a polypeptide comprising an amino acid sequence corresponding to autotaxin, or a fragment thereof having at least 5 amino acids.

5 2. The DNA segment according to claim 1, wherein said DNA segment encodes the amino acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38 through SEQ ID NO:52, SEQ ID NO:66
10 and SEQ ID NO:69.

3. An isolated polypeptide comprising an amino acid sequence corresponding to autotaxin, or a fragment thereof having at least 5 amino acids.

15 4. The polypeptide according to claim 3, wherein said amino acid sequence comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO:36, and SEQ ID NO:38 through SEQ ID NO:52, SEQ ID NO:67 and SEQ ID NO:69.

20 5. An isolated polypeptide bound to a solid support, comprising an amino acid sequence corresponding to autotaxin, or a fragment thereof having at least 5 amino acids.

25 6. The polypeptide according to claim 5, wherein said polypeptide comprises the amino acid sequence selected from the group consisting of the SEQ ID NO:1 through SEQ ID NO:11, SEQ ID NO:26 through SEQ ID NO:34, SEQ ID NO: 36, SEQ ID NO:38 through SEQ ID NO:52., SEQ ID NO:67 and SEQ ID NO:69.

30 7. A recombinant DNA molecule comprising a vector and the DNA segment according to claim 1.

8. A cell that contains the recombinant DNA molecule according to claim 7.

35 9. An antibody having binding affinity for autotaxin, or binding fragment thereof.

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10. A method of producing a recombinant autotaxin polypeptide said method comprising:
culturing a cell containing the recombinant DNA molecule of claim 7 under conditions such that the DNA segment is expressed, producing said polypeptide; and
5 isolating said polypeptide.
11. A method of purifying the autotaxin peptide of claim 3, comprising the steps of:
i) collecting and concentrating supernatant from cultured A2058 human melanoma cells
10 whereby a first preparation of said peptide is produced;
ii) salt fractionating said first preparation to produce a second peptide preparation;
iii) isolating said peptide from said second preparation so that said peptide is obtained in
15 substantially pure form.
12. The method of claim 11, wherein said isolating step is effected by column chromatography.
13. An isolated DNA encoding an autotaxin protein or fragment thereof wherein said DNA includes a
20 nucleic acid sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:37 and SEQ ID NO:38.
14. The DNA segment according to claim 1, wherein said DNA fragment comprises any one of the SEQ ID NO:12 through SEQ ID NO:25, or SEQ ID NO:39 through SEQ ID
25 NO:52.
15. The DNA segment according to claim 13 wherein said DNA segment comprises any one of the SEQ ID NO:12 through SEQ ID NO:25.
16. An isolated polypeptide comprising an amino
30 acid sequence corresponding to autotaxin.
17. A polypeptide bound to a solid support and comprising an amino acid sequence corresponding to autotaxin.
18. A recombinant autotaxin polypeptide
35

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° according to claim 3.

19. An isolated polypeptide according to claim
3 having cell motility activity.

5

10

15

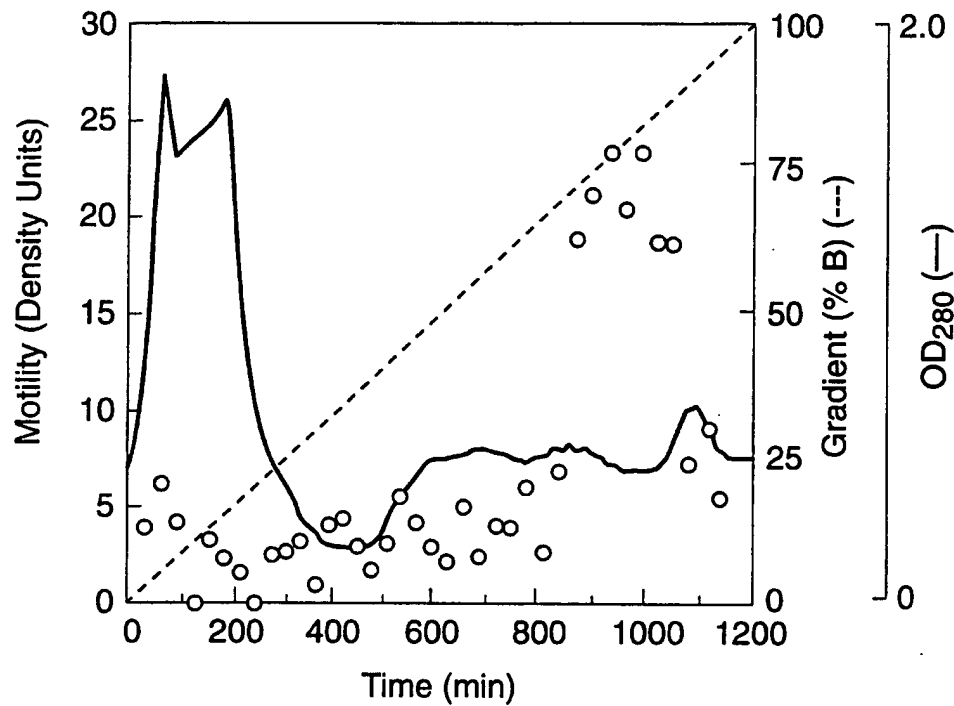
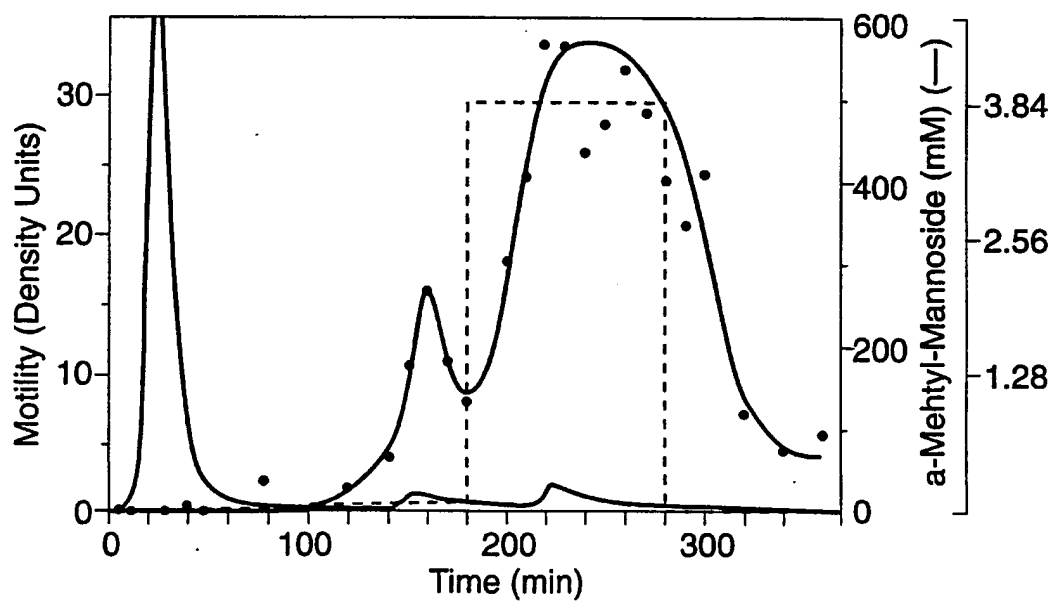
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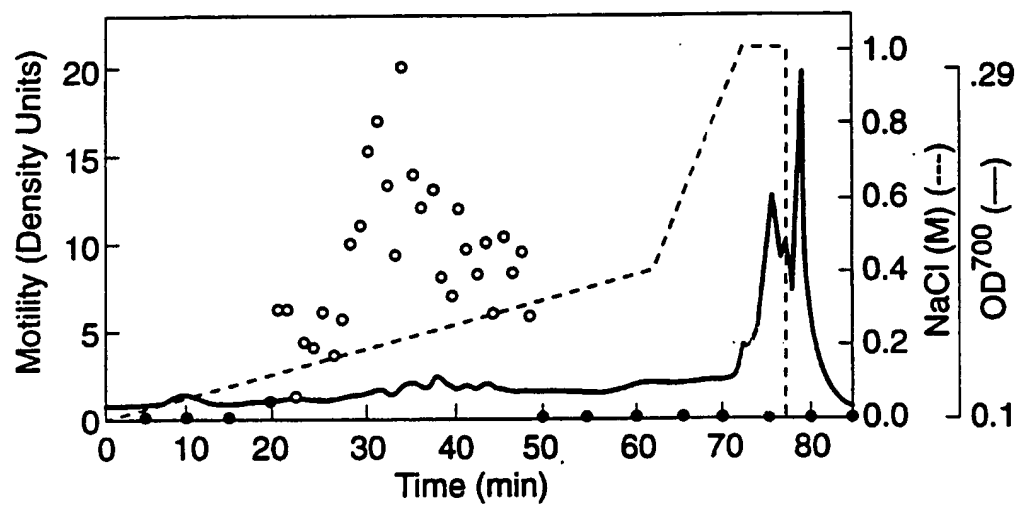
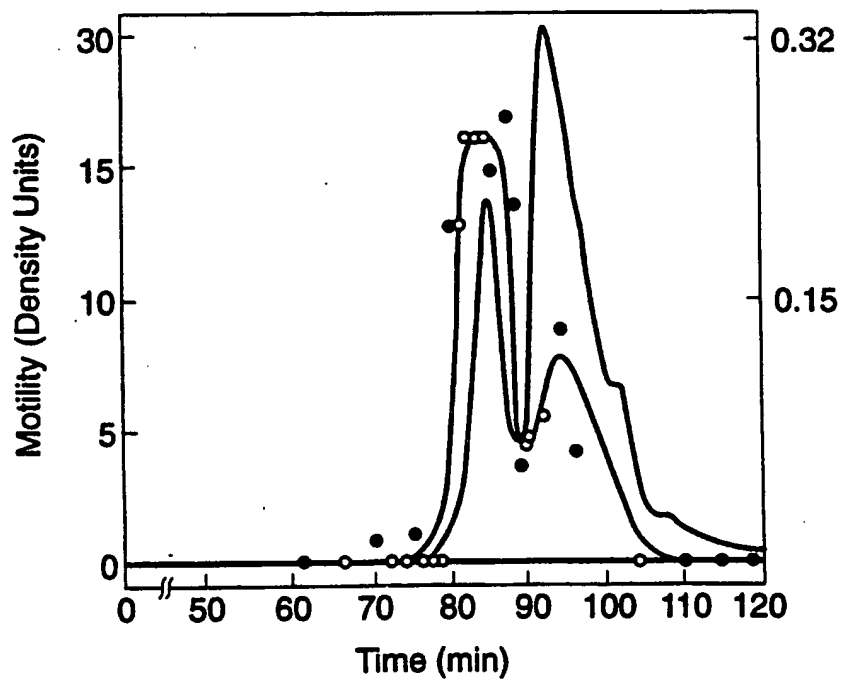
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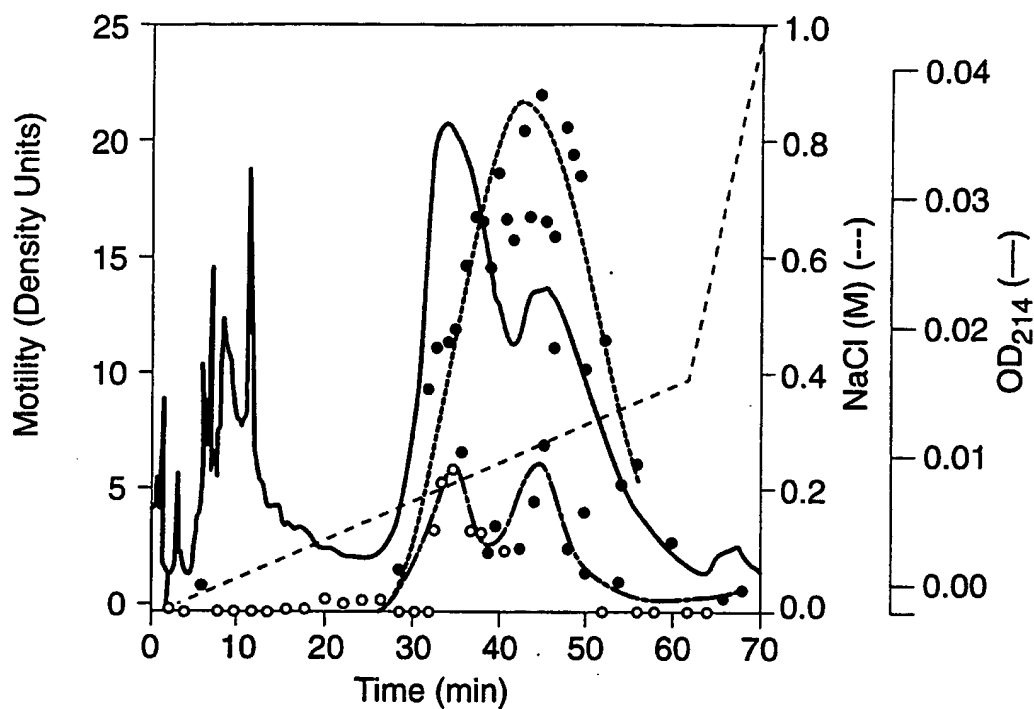
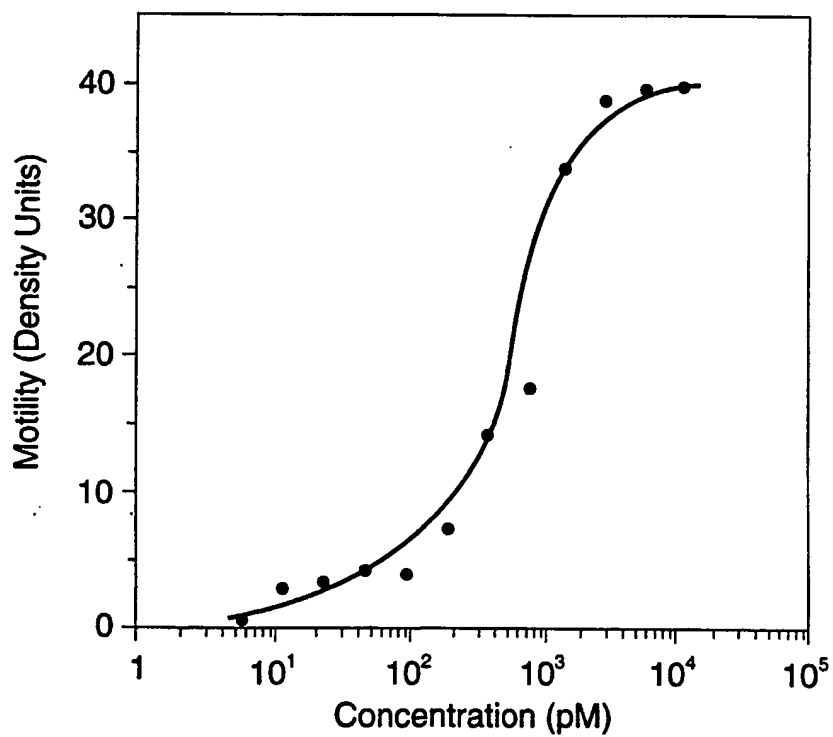
1/15

FIG. 1**FIG. 2**

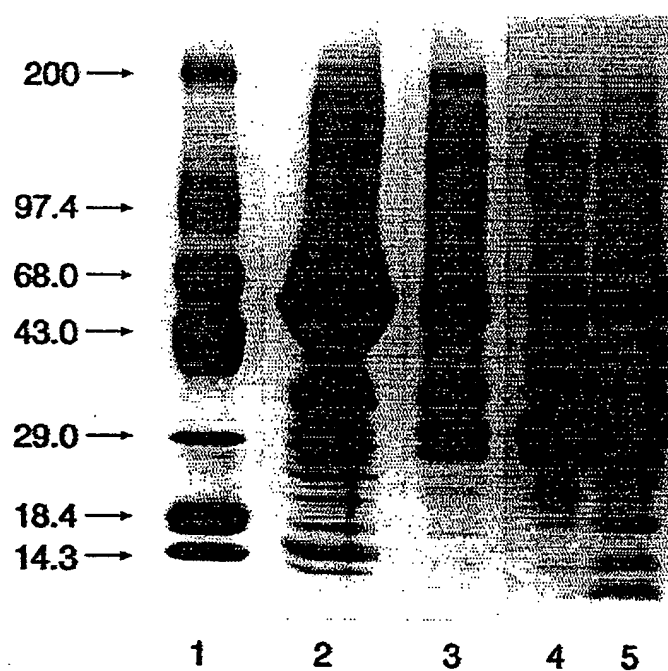
2/15

FIG. 3**FIG. 4**

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FIG. 5**FIG. 8**

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FIG. 6A

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FIG. 6C

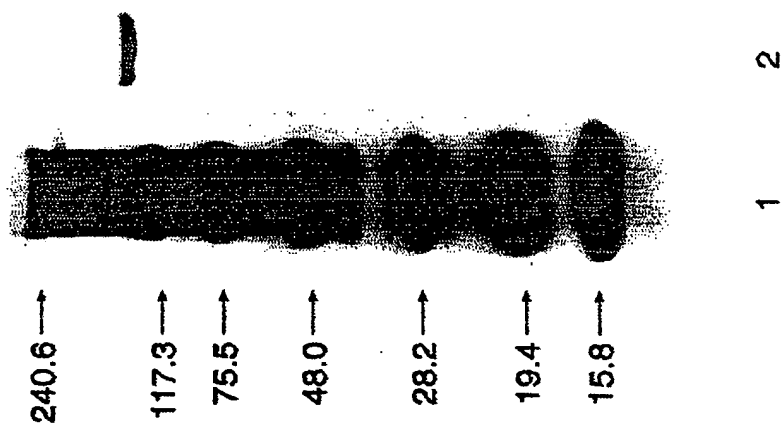
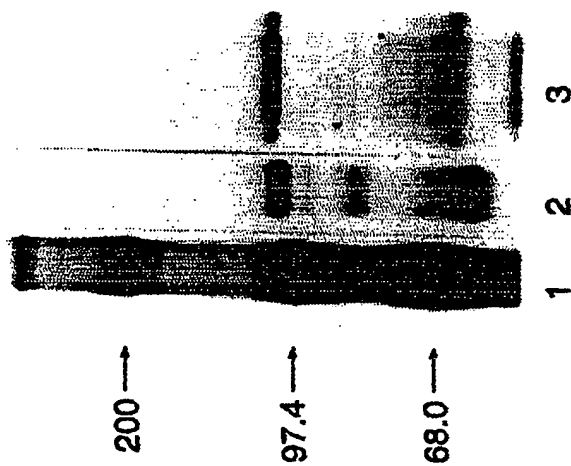


FIG. 6B



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FIG. 7

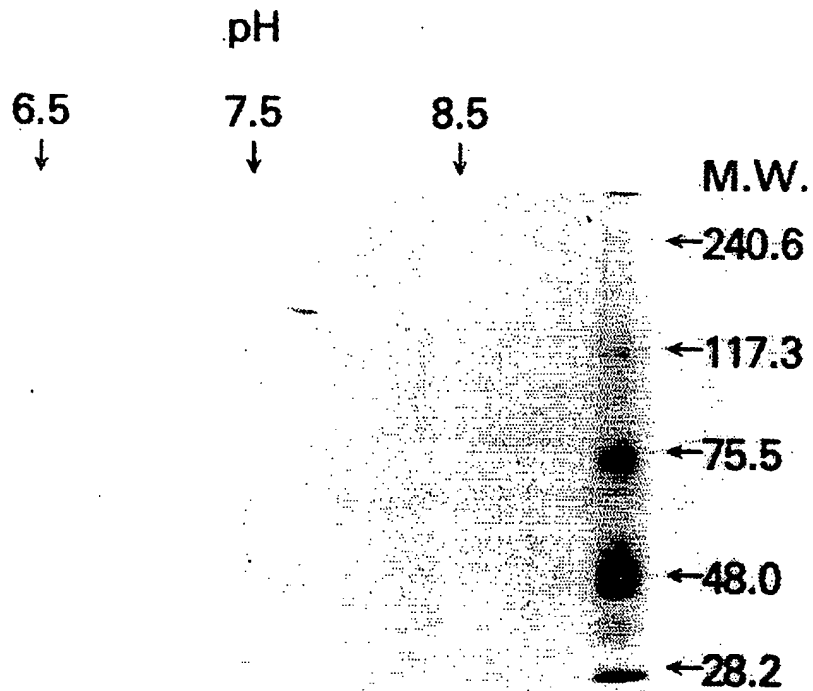
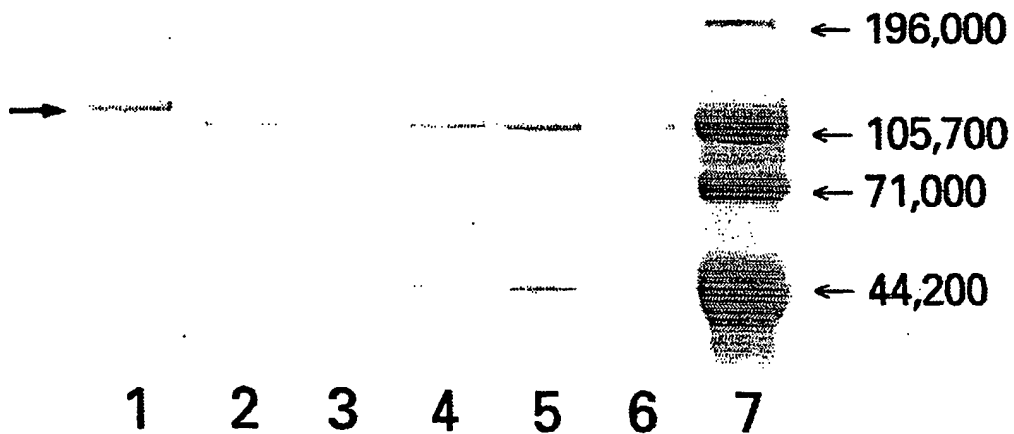
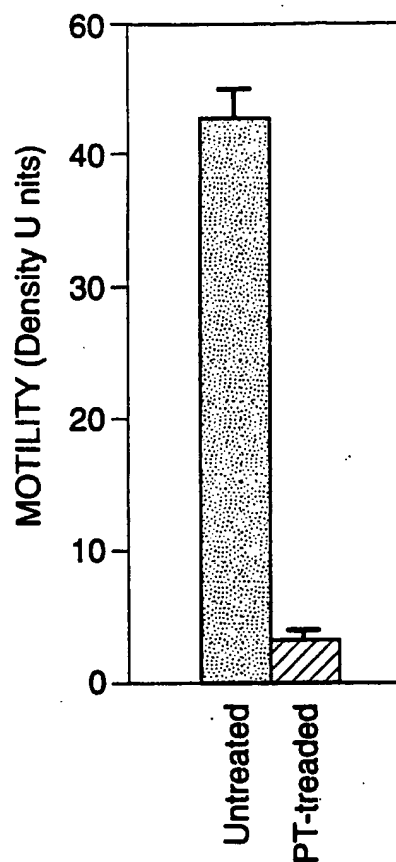


FIG. 16

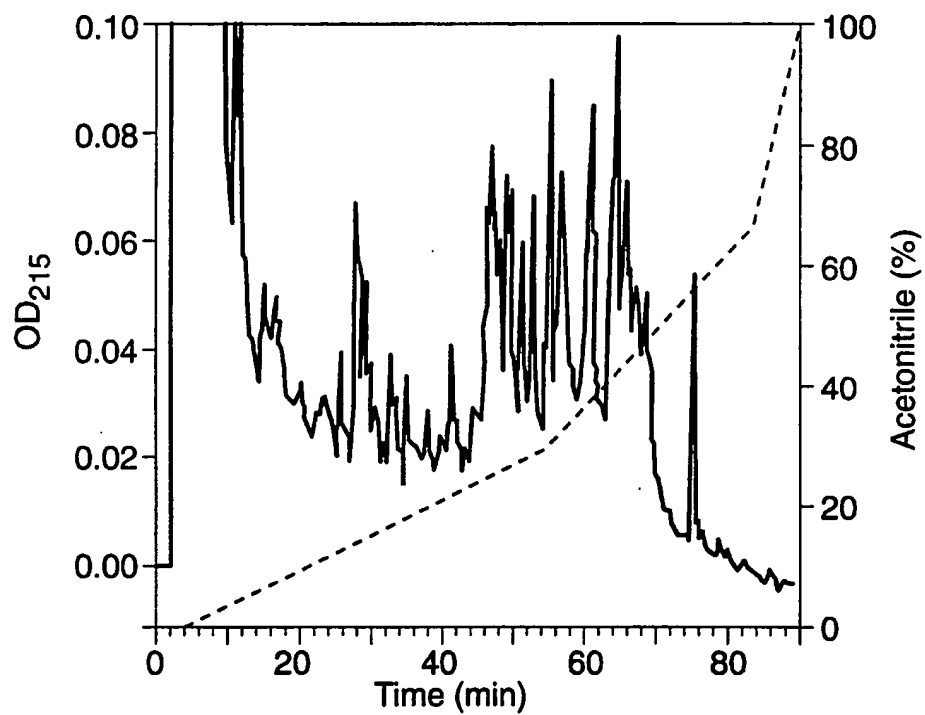


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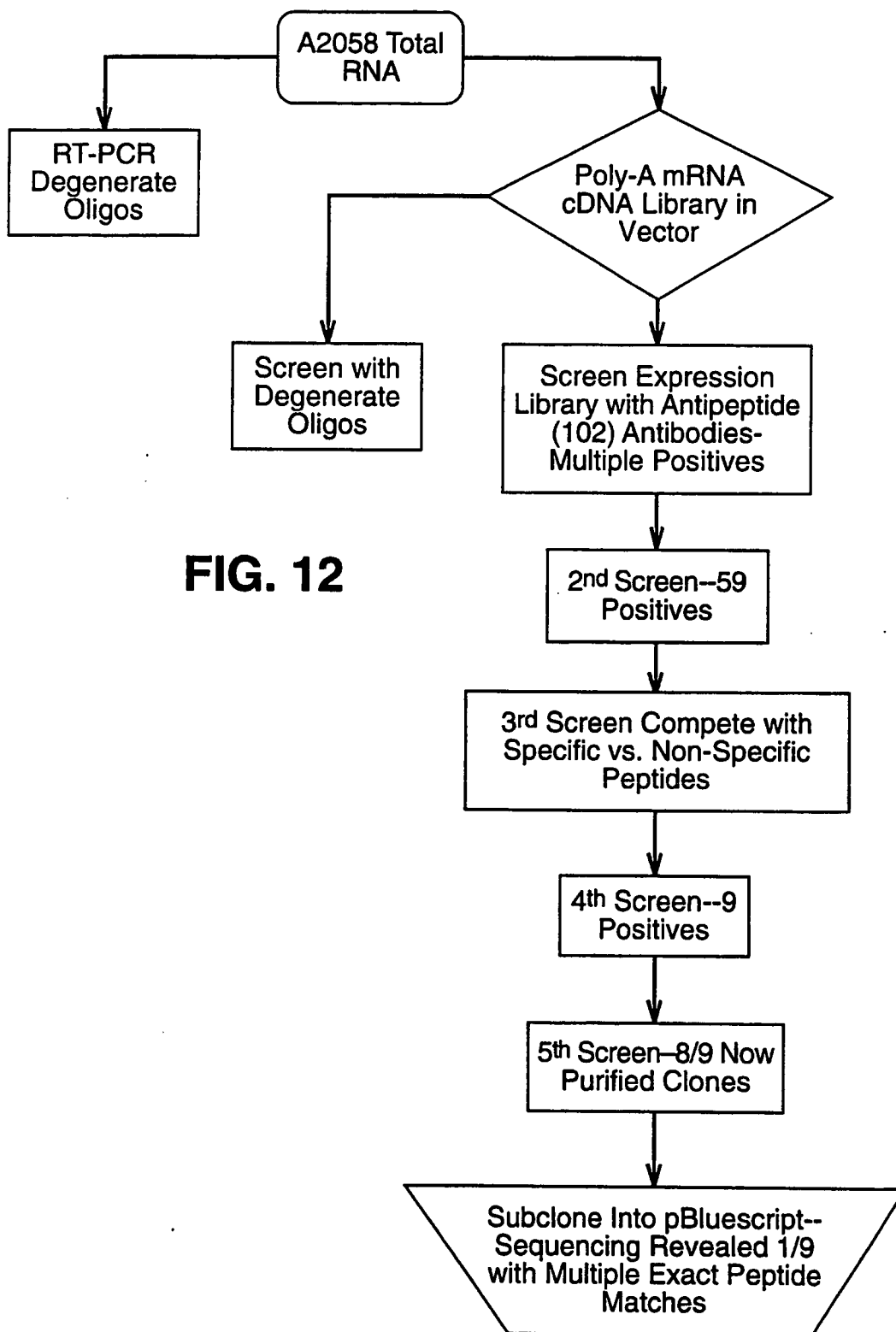
FIG. 9**FIG. 10**

		Upper Walls		
		0	0.01%	0.1%
Lower Walls	0	4.8 ± 0.3	13.7 ± 0.8	33.8 ± 1.6
	0.01%	45.4 ± 4.0	39.3 ± 2.6	34.9 ± 1.4
	0.1%	75.6 ± 1.8	58.3 ± 3.1	41.0 ± 3.4

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FIG. 11

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**FIG. 12**

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FIG. 13

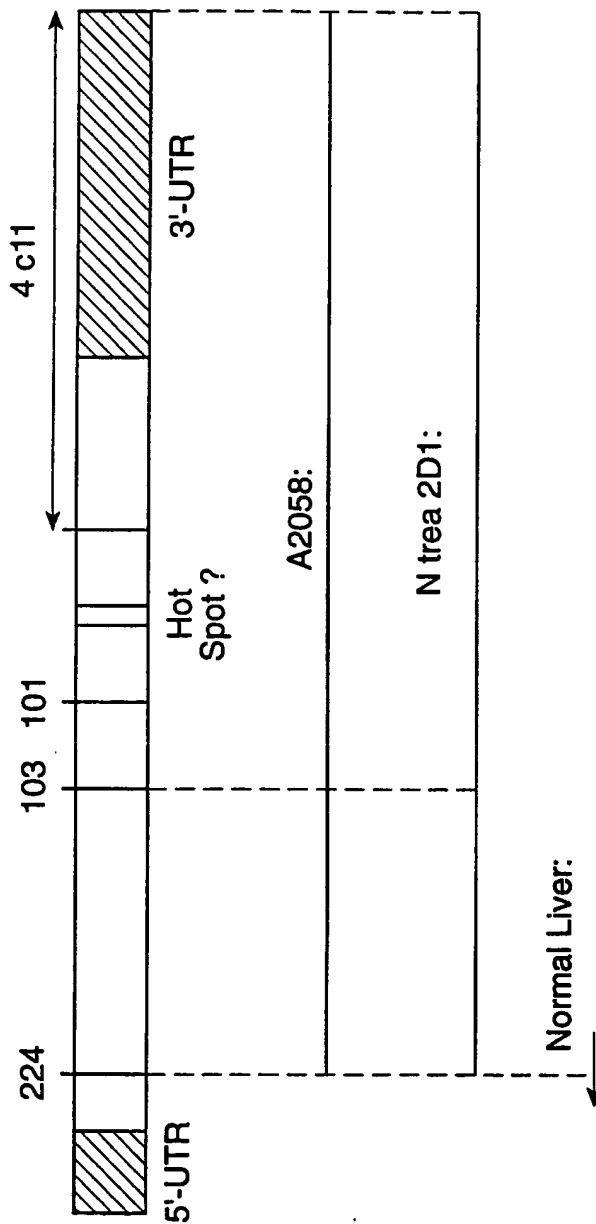
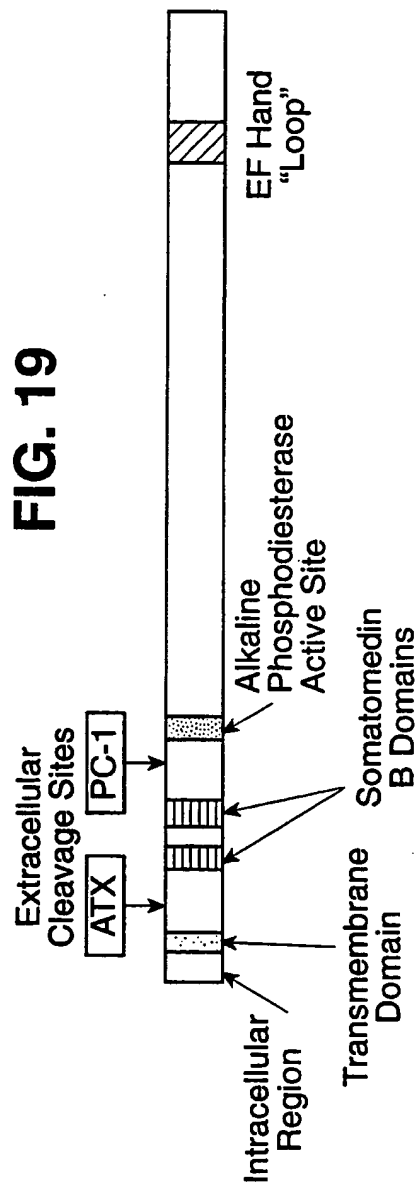
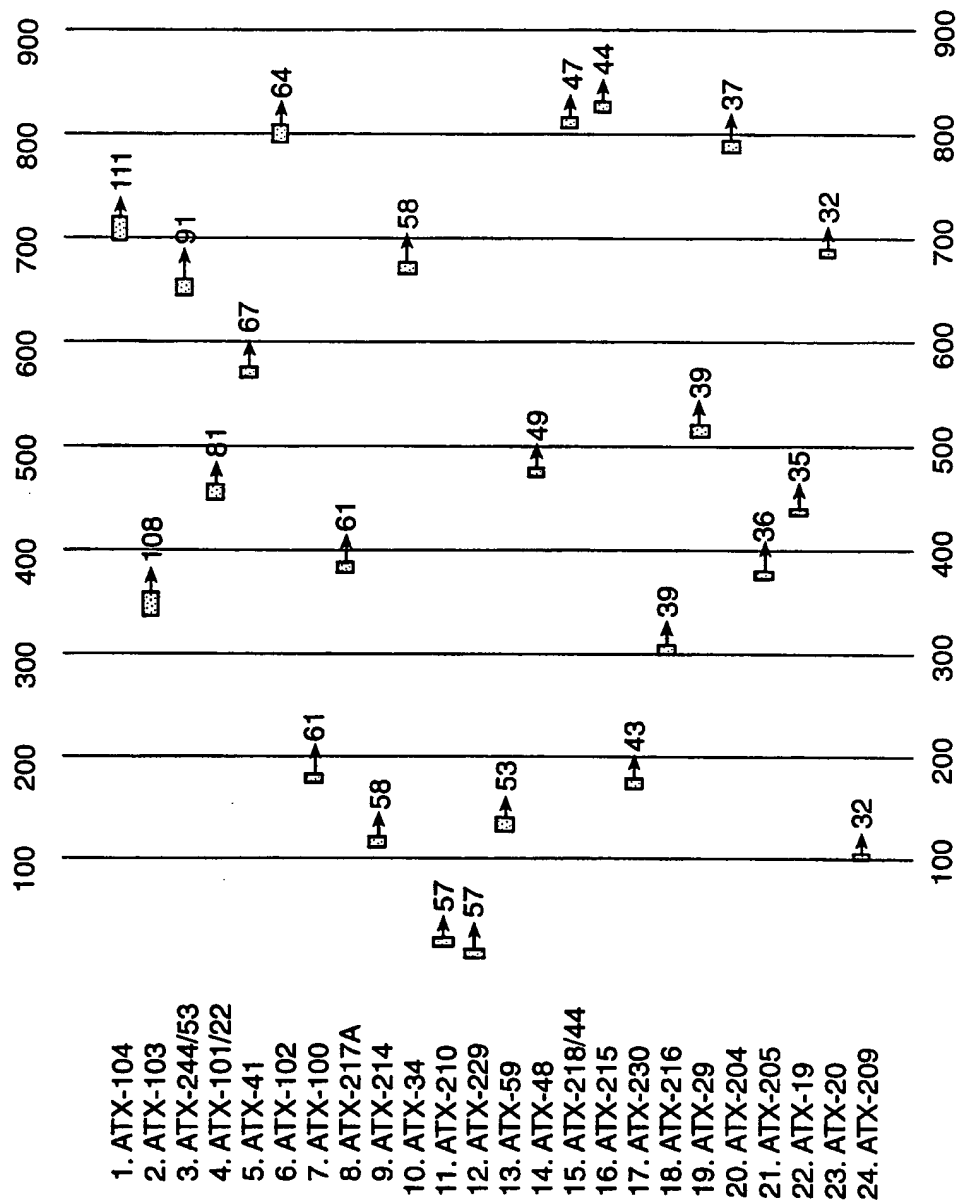


FIG. 19

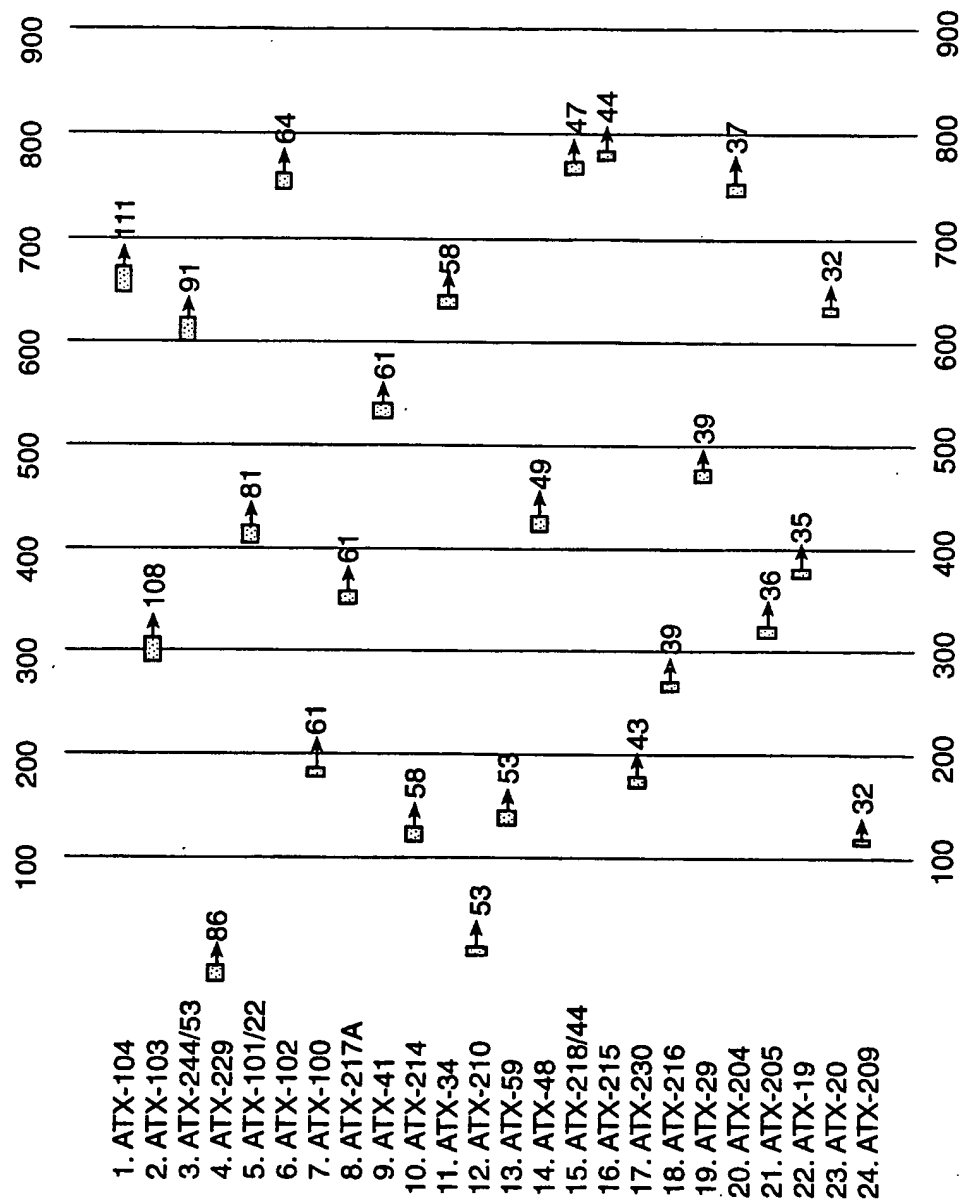


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FIG. 14

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FIG. 15



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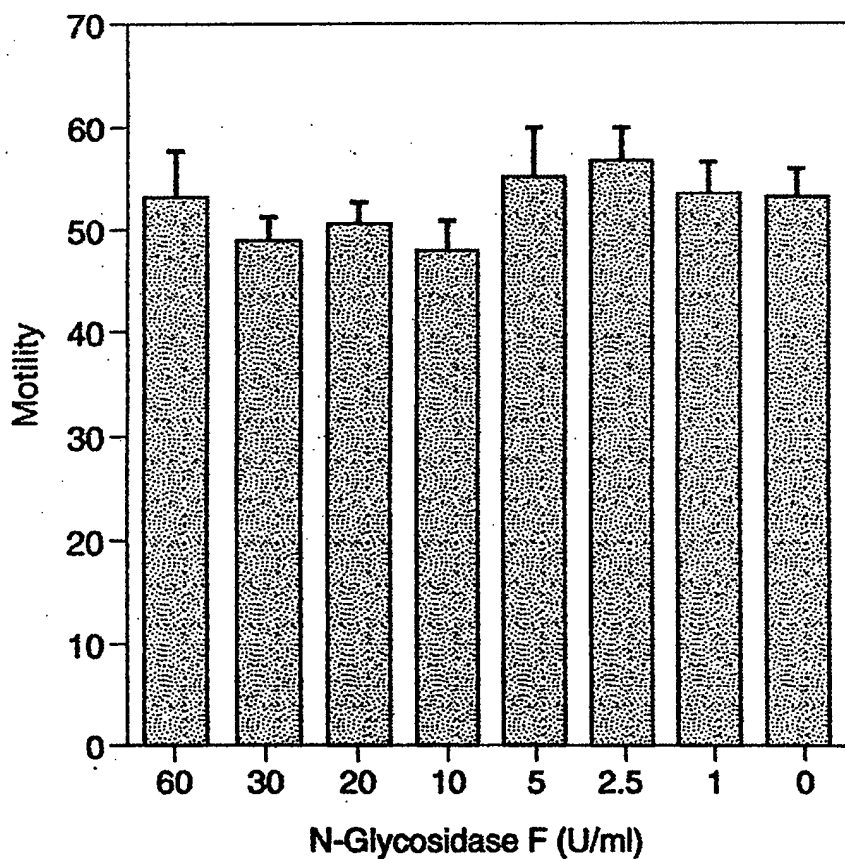
FIG. 17A**FIG. 17B**

FIG. 18A

hATX	MARRSFQSCQIIISLFTFAVGSVICLGFTAHRIKRAEGWEGPPTVLSDSPWTNISGCKGRCFELQAGPPDCRDNLCKSYTSCCHDF	90
hPC1	MDVGEEPLEKAARARTAKDPNTYKVLVLVLCVLTIL.....GCIFG.....LKPSCAKEVK.SCKGRCF...ERTFGNCRCDAAACVELGHCCLDY	84
hATX	DELCLKTARGWECTKDRCGEVRNEENACHCSEDCLARGDCCTNYQVCKGESHWDDECEIIKAAECFAGFVRPPLIIFSVDGFRASYMKGSKVMPNIE	190
hPC1	QETCIEPIHWTCNKFRCGEKRLTRSLCACSDDCDKDKGCCINYSVCQGEKSWVEEPCEINEPQCPAGFETPTLLFSLDGFRAEYLHTWTGGLLPVIS	184
hATX	KLRSCGTHSPYMRPVYPTKTFPNLYTLATGLYPESHGIVGNSMYDPVDFATFHLRGREKFNHRWGGQPLWITATKQGVKAGTFFWS.....	272
hPC1	KLKKGCTYTKMRPVYPTKTFPNHYSIVTGLYPESHGIIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIFPDI	284
hATXVWIPHERRILTILRWLTPDHERPSVYAFYSEQPDFSGHKYGFPGPESSYGSPTTPAKRPKRKVAPKRRQERPVPAPKRRRKIIHRMDHYAAET	372
hPC1	YKMYNGSVPFEEIILAVLQWLQPKDERPHFYTLYLEEPDSSGHSYGPVSSE.....	336
hATX	RQDKMTNPLREIDKIVGQLMDGLKQLKLRRCVNVIFVGDHGMEDVTCDRTEFLSNYLTNVDDITLVPGTLGRIR.SKFSNN.AKYDPKAIIANLTCKKPD	470
hPC1VIKALQVRVDMVGMWMLMDGLKELNLHRCNLILTSDHGMEOGSCCKYIYLNKYLGDVKNIKYVPAARLRPSDVPDKYSPNVEGIARNLSCREP	432
hATX	QHFKPYLKOHLPKRLHYANNRRIEDIHLLVERRHWVARKPLDVYKPKSGKCFQGDHGFNKNVSNMOTVPVGYGPTFKYKTKVPPFENIELYNVMCDLIG	570
hPC1	QHFKPYLKHGLPKRLHFAKSDRIEPLTFYLDPOWQALNPSE..RKYCGSGF.....HGSNDVFSNMQALFVGYGPGFKHGIEADTFENIEVYNLMCDLLN	526

FIG. 18B

[illegible]